THE JOURNAL OF
PEDIATRICS

MAY 1961 *Volume* 58 *Number* 5

Symposium on the nephrotic syndrome: Introduction

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"THAT a certain collocation of functional disturbances having something to do with the kidney" may be designated by the "relatively harmless adjectival form" "nephrotic syndrome" rather than the noun "nephrosis," which implies a clinical or morphologic form of renal disease, recently was emphasized by Jean Oliver. Further, he continues inimitably, these terms embrace an "omnium-gatherum of renal enigmas."[1]

The task of challenging the bogey of modern nephrology was accepted by the authors of the symposium which follows. In its manifestations, familiar to most pediatricians, the nephrotic syndrome provokes parents to ask: "What causes it; is it nephrosis or nephritis; why is my child so swollen; what about special treatments like diet, blood transfusions, steroids?" Ultimately, when confidence is attained, the most important question of all is asked: "Will he get well?" Not only must the physician, as oracle, answer these questions accurately and in patient detail, but his answers must be tempered with a sympathetic understanding of the emotional consequences of the disease, and of his answers, for both patient and parent. It is hoped that some perspicuous solutions to the enigmatic "omnium-gatherum" will be found in the following papers.

It should be emphasized that this Symposium is not intended to represent a review of general opinion pertaining to each of the many facets of the nephrotic syndrome; rather, the papers represent viewpoints

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derived from both clinical and research activities in selected, important areas by experts concerned with the nephrotic child. Nor is this Symposium designed to present oversimplifications of all the complicated problems relating to this disease. It is hoped that the careful reader will discover the potential usefulness of the experimental models of the nephrotic syndrome illustrated by the investigations on animals, and become reoriented to the complications of pathologic interpretations imposed by development of a new instrument, the electron microscope—a device which may magnify insights into morphologic structure but by its very nature may also magnify errors of interpretation. Surviving these sallies, the reader may venture into the brackish waters and discover that in this disease, at least, neither sodium retention nor protein depletion per se affords a complete explanation of the pathogenesis of edema. The physician may find the report on physiologic considerations in therapy of the nephrotic syndrome of particular, practical interest.

Finally, the patient, rather than the disease, is brought into focus with the last paper in this Symposium. A sensitive introduction to the impact of this unpleasant disease upon the patient, his parents, his family, and his society should provide some insights for the thoughtful physician. Perhaps with this background, the pediatrician will find new strengths when approached by the challenge of the nephrotic syndrome.

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Pathogenesis of the nephrotic syndrome: Considerations based on clinical and experimental studies

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THE traditional confusion of terminology and classification of the nephrotic syndrome dates back to the original work of Mueller[1] and Fahr[2] who first described the separation of "nephrosis" from the nephritic diseases. The views held today differ from the previous ones and have led to a conception which seems to be better adapted to actual bedside experiences.

DEFINITION OF THE NEPHROTIC SYNDROME

The diagnosis of nephrotic syndrome is accepted today if massive proteinuria, hypoproteinemia, chiefly due to hypoalbuminemia, hyperlipemia, and anasarca are simultaneously noted. Although massive edema and ascites may be absent in rare instances throughout the course of the disease,[3] it is usually absent only during periods of remissions. If, in addition to the above noted symptomatology, the light microscope reveals thickened, frayed glomerular basement membranes,[4] and if the electron microscope indicates that this lesion is chiefly due to an alteration of the epithelial layers of the basement membrane, specifically to a fusing or smudginess of the podocytes,[5]* the requirements for the diagnosis of nephrotic renal disease are fulfilled.

EXPERIMENTAL NEPHROSIS

Many experimental data have been accumulated during the last 12 years, which will be referred to frequently in the following discussion. A short summary of the various forms of experimentally produced nephrotic renal diseases, therefore, shou'd be useful.

NEPHROTIC SYNDROME PRODUCED IN RATS BY CHEMICAL AGENTS

In 1955 Frenk and colleagues[6] reported that an aminonucleoside derived from

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The experimental work quoted in this communication was supported by United States Public Health Grant (A-44), by The American Heart Association, The Cleveland Heart Society, and The Northern Ohio Chapter of the National Kidney Disease Foundation.

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*See paper by Vernier, Worthen, and Good, this Symposium, page 620.

Table I. Natural course of aminonucleoside nephrosis in rats observed for 8 to 15 months

No. of rats	*Mode of aminonucleoside administration*	*Dose per 100 Gm.*	*Spontaneous remission*			*Outcome*
			No. of rats	*Onset after first injection*	*Duration (months)*	
5	Subcutaneous	0.3 c.c. of 0.5 Gm. per cent solution every day × 13	3	5 weeks to 5 months	1 to 3	1 cure 2 chronic disease without renal failure 2 chronic disease ending in renal failure
17	Intravenous	10 mg. but not more than 20 mg. × 1	5	3 weeks to 3 months	1 to 5	0 cures 9 chronic disease without renal failure 8 chronic disease with renal failure

puromycin induced severe nephrotic renal disease in rats. The chemical structure of this compound (6-dimethylamino-9-[3′-amino-3′ desoxy- -D-ribofuranosyl] purine) and its similarity to adenosine are shown in Fig. 1. The enteral or parenteral administration of this substance always produces severe nephrotic renal disease in rats. A latent period of 5 to 11 days, depending on the mode of administration, is regularly noted.[7] Spontaneous subsidence of this disorder occurs occasionally, but more commonly the disease goes on for many months, leading to death from renal failure which was assumed when progressive azotemia, increased blood creatinine values, and hypertension were noted (Table I). In these instances the histologic studies, obtained in almost all animals at time of death, revealed severe proliferative changes and glomerular fibrosis. It is of considerable interest to note that this aminonucleoside does not produce nephrotic renal disease in mice, rabbits, guinea pigs, or dogs,[7] even though the unaltered compound is excreted in the urine of these animals at approximately the same rate as in rats.[8] The regularity with which a well-defined latent period is observed suggested the possibility that an immunochemical mechanism might be involved in its action. The observation that ACTH, cortisone, x-ray radiation, and the intravenous injection of serum obtained from an animal that had just developed proteinuria did not affect the latent period or the course of this experimental disease refutes this possibility. A direct chemical action is thus more likely but still awaits clarification. The close relationship of this compound to adenosine suggests the hypothesis that the aminonucleoside acts as an antimetabolite in the metabolic pathways of certain nucleic acids in the kidneys.

Trimethadione (Fig. 2), chemically unrelated to the aminonucleoside derived from puromycin, has been described to produce in rare instances a nephrotic syndrome in man.[9] It has recently been shown that 11 of 22 rats that were given this compound by the oral route daily for as long as 18 months developed nephrotic renal disease.[10] In the 11 rats that had been treated for more than 6 months but had not developed a nephrotic syndrome (no proteinuria, hypoalbuminemia, or hyperlipemia) slight, but definite histologic alterations of the glomerular basement membrane also were noted. The incidence of experimental trimethadione nephrosis in rats is very much higher than the incidence in man. Among the innumerable patients treated for petit mal with dione derivatives, not many more than a dozen instances of Tridione nephrosis have been reported in the literature.[11] It has to be realized, however, that the dose given to rats was, in relation to body weight, 4 to 5 times as great as the one used in man. None of the rats in which the administration of Tridione was discontinued after proteinuria had persisted

for several weeks showed evidence of spontaneous subsidence. This is in contradistinction to experiences in man, even though some cases have been reported[12] that did not subside after Tridione medication was discontinued.

NEPHROTIC SYNDROME PRODUCED IN RATS BY ANTIBODIES TO KIDNEY PROTEINS

It was shown in 1951[13] that the intravenous injection of anti–rat kidney sera obtained from rabbits induces a nephrotic type of renal disease in rats that closely simulates the pathologic changes, clinical course, and outcome of the disease observed in infants and children. When heteronephrotoxic sera were obtained from rabbits, no latent period was noted. In fact, evidence of glomerular disease was obtained by histologic examination within one hour after the injection (Fig. 4, *A*).[14] When heteronephrotoxic sera were obtained from ducks, a definite latent period (4 to 21 days) was observed quite regularly.[15] The length of the latent period has some direct relationship to the potency of the sera since a few very potent duck sera induced immediate renal disease.[15]

Under certain experimental conditions approximately 30 per cent of the rats showed spontaneous and complete recovery from experimental nephrosis produced by anti-kidney sera from rabbits. The others developed a chronic renal disease that lasted for 1 or 2 years or that led to an earlier death from renal failure. The incidence of progressive renal failure could be varied experimentally almost at will. The age of the sera, the species of recipient animals, the species from which anti–rat kidney serum was obtained, and chiefly the protein intake of the injected rats were decisive factors in determining the outcome of the disease.

Although these studies showed that heteronephrotoxic antibodies produced renal injury with resulting renal disease of a nephrotic type, it had not been possible until recently to show that within the same species kidney proteins could be rendered antigenic. This has been demonstrated recently in rats by repeated intraperitoneal injections of Freund's adjuvant mixed with saline suspensions of renal tissue obtained from rats.[16] Six of 7 rats subjected to unilateral nephrectomy and injected 5 to 11 weeks later with adjuvant and kidney suspensions prepared from their own previously removed kidneys developed severe nephrotic renal disease after 2 to 8 treatments (Fig. 3). With the use of Ouchterlony's double diffusion agar plate method, precipitating antibodies to kidney proteins in sera of these animals have been shown to be present for possibly 4 weeks prior to and for up to 8 weeks after onset of proteinuria.[17] With the onset of proteinuria a decrease in complement activity also was noted. By means of Boyden's technique with Stavitsky's modification,[18] hemagglutination titers were less regularly, but, nevertheless, definitely obtained in sera of some of these animals. It is assumed that these renal auto- and/or isoantibodies may be responsible for

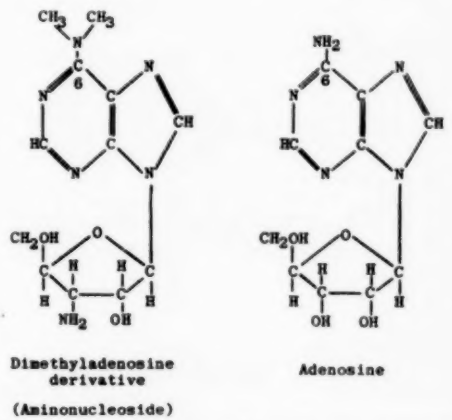


Fig. 1. Comparison of the chemical structure of the aminonucleoside derived from puromycin with adenosine.

3-5-5-trimethyloxazolidine-2-4-dione

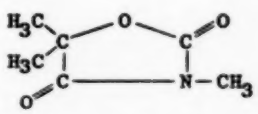


Fig. 2. Chemical structure of trimethadione (Tridione).

the ensuing renal disease, but the proof for this is still lacking.

PATHOGENETIC CONSIDERATIONS OF THE DISEASE IN MAN

It is well known that in man a multitude of different etiological agents may also induce a nephrotic syndrome. They are:

1. Systemic diseases: amyloidosis, diabetes mellitus, systemic lupus erythematosus, syphilis.

2. Renal diseases: chronic glomerulonephritis, acute poststreptococcal glomerulonephritis, renal vein thrombosis.

3. Chemical agents: trimethadione, Paradione, penicillamine, gold salts, bee stings, poison oak, mercury.

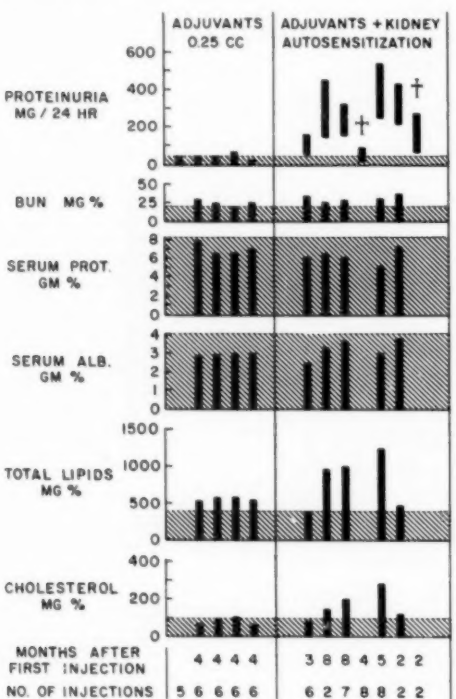


Fig. 3. Proteinuria, slight hypoproteinemia, hyperlipemia, and hypercholesterolemia in 6 of 7 rats (right) treated with Freund's adjuvant and rat kidney suspensions obtained by unilateral nephrectomy 5 to 11 weeks prior to first intraperitoneal injection.

4. Etiology unknown.

Even though some of the substances listed (bee stings, exposure to poison oak, and mercury) cannot be considered without some reservations, the remaining ones have been established as causative agents beyond reasonable doubt. The nephrotic renal disease, commonly seen in infants and children, of which the etiology is unknown, belongs in Group 4. Based on bacteriologic and serologic studies one can state with much assurance that a preceding streptococcal infection, so well established in the etiology of acute glomerulonephritis,[19] does not play a role in the production of the nephrotic syndrome of infants and children. Any etiological connection to other coccal or viral infections has not been established, although this possibility is under reinvestigation.[20] The aggravation of the nephrotic disease process by an intercurrent infection is well recognized but obviously has no relationship to the original causative agent.

A much discussed and, at present, quite prevalent inclination is to favor an immunochemical etiology, which would place the nephrotic syndrome as seen in infants and children in the group of "auto-immune diseases." This concept is supported by the experimental production of the nephrotic syndrome in rats by the injection of anti–rat kidney sera obtained from rabbits and lately by the experimental production of renal autoantibodies in rats with the subsequent development of nephrotic renal disease. From these experimental studies the only permissible implication is that certain forms of renal antibodies can produce a nephrotic type of renal disease similar to the human disease. Available evidence, however, is at best compatible with such a hypothesis but thus far has failed to show that antibodies to kidney proteins are demonstrable in man. The decreased complement activity of sera of nephrotic patients[21, 22] and the increased amounts of globulins demonstrable in the glomerular structure of nephrotic kidneys[23] provide only suggestive evidence. Hemagglutination titers and the demonstration of precipitating antibodies in the sera of neph-

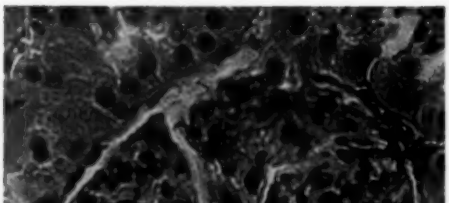
rotic children reported by Liu and McCrory[24] also were obtained in patients with nonrenal acute infectious diseases, rheumatic fever, rheumatoid arthritis, and lupus erythematosus and may be explained by acute phase substances rather than by true antibodies. Thus the etiology of the nephrotic syndrome of "unknown etiology" in man still remains unclarified. It could be assumed just as well that the disease is caused by the action of a chemical substance thus far unknown.

THE GLOMERULAR LESIONS OF THE NEPHROTIC SYNDROME

Whatever the etiological agent of this disorder may be, it has during recent years been well established that the primary site of the disease process is in the glomerulus. The old concept of Mueller and Fahr, which separated nephritic renal disorders as inflammatory lesions of the glomeruli from nephrotic forms of renal diseases as primarily degenerative processes in the tubules, is no longer tenable. The early lesions of the disease have been established in man by serial studies of discrete renal functions[25] and more recently by electron microscopic studies of renal biopsies obtained in early stages of the disease.[5] The latter studies indicate that the glomerular lesion of nephritic diseases is different from the one noted in the nephrotic syndrome. In the former, endothelial structures seem to be primarily affected, whereas, in the nephrotic syndrome the first and most marked histologic lesion is localized in the epithelial layer of the glomerular basement membrane. In the evaluation of histologic studies it is well to realize that sequential examinations may be necessary to make firm distinctions between nephritis and nephrosis since the histologic responses of the kidney to different injurious agents may be quite similar. An instructive experience has been gained in this respect from serial histologic studies of the kidneys of rats injected with anti–rat kidney sera obtained from rabbits. Within the first 8 hours after the injection of such sera the glomeruli may show an intense polynuclear invasion, indistinguishable from the glomerular changes noted in acute glomerular nephritis (Fig. 4, *A*).[14] Glomeruli of rat kidneys studied at a later date no longer recorded any of these changes but showed the alteration of the glomerular basement membrane commonly associated with nephrosis (Fig. 4, *B*). One and the same etiological agent, a heteronephrotoxic antibody, thus elicited the spectrum of histologic lesions characteristic of "acute glomerulonephritis," "nephrosis," and finally "chronic glomerulonephritis" (Fig. 4, *C*).[26]

HEMATURIA IN THE NEPHROTIC SYNDROME

After it became established that the primary site of the disease process in the nephrotic syndrome is in the glomerulus, it became less objectionable to accept hematuria[53] as one of the symptoms that may occur in the syndrome. It is less frequently noted than in nephritic diseases, but, even in macroscopic form, may be compatible with a diagnosis of the nephrotic syndrome. In our experience gross hematuria has been detected in approximately 7 per cent of nephrotic children.[27] The urine in such cases has a bright red or pink color, different from the brown to purplish tinge that usually characterizes the grossly hematuric urines of nephritic patients. The cellular elements do not settle as rapidly in the former when urine is permitted to stand at room temperature. The difference in color seems to be due to the fact that erythrocytes are hemolyzed to a greater extent in nephritic urine. The usually acid urine then readily converts the larger amounts of hemoglobin into brown acid hematin. This was established by the finding of a greater number of shadow cells derived from red blood cells in nephritic urines than in nephrotic ones. To a certain extent, the escape of red blood cells into the urine may be connected with the intensity of proteinuria, as suggested by the observation that the intravenous infusion of large amounts of plasma albumin in nephrotic children not infrequently produces, or increases, an already present hematuria.[28]



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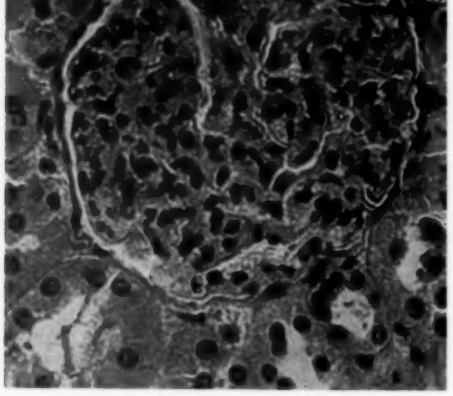

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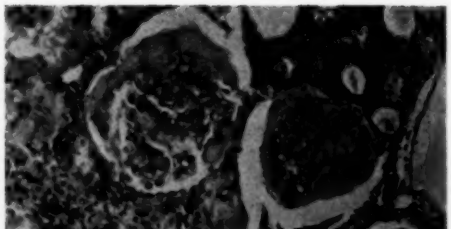

C

Fig. 4. Histologic alteration of glomeruli of rats injected with rabbit anti-rat kidney serum. *A*, Obtained 1 hour after injection. *B*, Obtained 7 days after injection. *C*, Obtained 11 months after injection.

PATHOGENESIS OF PROTEINURIA AND HYPOPROTEINEMIA

Inasmuch as the pathogenesis of proteinuria and hypoproteinemia in the nephrotic syndrome is discussed in other papers of this symposium, the considerations here will be restricted mainly to our studies on rats injected with nephrotoxic sera and on urines obtained from nephrotic children.

The extensive studies of Lauson[29] and Slater[30] seem to indicate that proteinuria in nephrosis derives chiefly from a leakage of unaltered plasma proteins through the glomerular basement membrane. The hypothesis that a primary alteration of plasma proteins accounted for the excretion of large amounts of proteins into the urine has not been supported by immunochemical studies of the proteins lost in the urine.[31] The concept of nephrosis as a "diabetes albuminuricus" of extrarenal origin thus has been abandoned. However, the assumption that hypoproteinemia is due exclusively to the loss of plasma proteins in the urine no longer seems to be satisfactory. Gitlin and associates[32] indicated that in certain stages of the disease in children an impressive rate of protein catabolism has to be taken into account. In earlier studies of changes in rats occurring during the first few hours after the injection of nephrotoxic sera, it was noted that hypoproteinemia developed in 10 of 30 rats at a time when the amounts of proteins

excreted in the urine were minimal and could not account for the observed depletion of plasma protein concentration.[14] It is still believed that proteinuria largely accounts for the decrease in plasma protein concentration. In most instances of the nephrotic syndrome the plasma proteins are decreased chiefly because of diminished plasma albumin concentration; plasma globulins, however, are also found in varying amounts in the urine of nephrotic patients and may account for 20 to almost 70 per cent of the total daily proteinuria. This has been established rather recently by electrophoretic methods.[33] In the course of these studies, the contention by Blackman[34] in 1941, that patients with marked and consistent globulinuria have a poorer prognosis has been confirmed. If frequently observed, a loss of more than 2 Gm. of globulins in the urine of nephrotic patients within 24 hours or an albumin to globulin ratio in the urine of less than 1.1 was usually associated with the ultimate development of renal failure.

A plea for a more accurate terminology seems justified. The term "albuminuria," implying that all urinary proteins are albumin, should be abandoned and be replaced by "proteinuria."

PATHOGENESIS OF NEPHROTIC HYPERLIPEMIA

The hyperlipemia detected in nephrosis in the human and in all experimentally produced forms in rats examined to date always involves all lipid constituents. Neutral fat, fatty acids, and phospholipids (lecithin) are increased as markedly as cholesterol. In fact, the evidence indicates that fatty acids start to increase sooner after onset of the disease than does cholesterol.[14] Friedman and Byers[35] believe that the cholesterol concentration may increase only secondarily to and as a consequence of the rise in fatty acid concentration. Fat droplet deposition in renal tubules is believed to result from tubular reabsorption of lipoproteins. There is no indication that this impressive histologic finding represents a specific intracellular disturbance of lipid metabolism in renal tubular cells. A similar explanation is accepted for the finding of double refractile lipid bodies in the urinary sediment of nephrotic urines. One visualizes the excessive amounts of lipoproteins excreted in the urine as derived from the large protein pool and, like other proteins, lost in the urine. It can be said with some certainty that hyperlipemia is essentially an endogenous metabolic error. The withdrawal of exogenous lipids from the diets of experimental animals[36] or of nephrotic patients[37] does not affect the degree of hyperlipemia appreciably. Hypoproteinemia, long held as the causative factor, has generally been abandoned. One of many reasons for this is the observation that hypoproteinemia does not precede but rather follows the development of hyperlipemia in experimental animals.[14] Rosenman, Friedman, and Byers[38] recently stated that hypoalbuminemia, rather than hypoproteinemia, was the responsible factor. Their interpretation was based on studies in which implantation of the ureters into the lower vena cava of nephrotic rats and/or intravenous infusion of albumin either inhibited development of or decreased existing nephrotic hyperlipemia. During the following year, however, it was shown that the intravenous injection of normal, protein-free urine decreased nephrotic hyperlipemia almost as markedly as protein-containing specimens obtained from nephrotic rats. It was also noted that the intravenous injection of dextran solutions decreased lipid concentration markedly in nephrotic animals.[39] Moreover, patients suffering from marked hypoalbuminemia not due to renal disease do not necessarily develop hyperlipemia.[40] Byers and co-workers[41] have subsequently suggested that the loss of large amounts of albumin in the urine plus the loss of a heparin-like clearing substance in the urine could lead to hyperlipemia; confirmation of this hypothesis is needed. The studies of Drabkin and Marsh[42] seemed to support the view that an increased mobilization of depot fats into the bloodstream could account for the hyperlipemia; however, depot fat analyses of animals after they had recovered from the acute phase of nephrotic renal disease which

frequently leads to a diminished food intake were found to be within the normal range.[43]

A stimulating hypothesis by Gitlin[44] involves hypoalbuminemia indirectly in the disturbed pathways controlling plasma lipid concentration. These data suggest that the low concentration of plasma albumin causes a greatly increased synthesis of low density or lipid rich beta lipoproteins as well as a decreased rate of conversion to the high density beta lipoproteins. As a result, there is an accumulation of the low density beta lipoproteins in the plasma with the accompanying lipids. The assumption that the marked depletion of plasma albumin frequently noted in the nephrotic syndrome is directly or indirectly involved in the production of the hyperlipemia does not agree well with the observations that: (a) there is no correlation between degree of hypoalbuminemia and severity of resulting hyperlipemia[37, 40] (hyperlipemia may persist for a long time after the plasma albumin concentration has returned to normal); and (b) in 40 per cent of children with acute glomerulonephritis the same type of hyperlipemia may be noted when hypoalbuminemia has been present for only a short time and to a much lesser degree.[45]

The role of the liver in the elicitation of nephrotic hyperlipemia also has been studied. Subtotal hepatectomy inhibits the development of hyperlipemia which follows the intravenous injection of nephrotoxic sera,[46] and lipid depots are decreased per unit of liver tissue in nephrotic rats.[43] When nephrotic hyperlipemia in rats is eliminated by administration of ethionine, the decreased liver lipids concomitantly[47] increase to normal values. Hepatomegaly is observed in forms of experimental nephrosis as well as in nephrotic children.[48]

At the moment, although many leads have been opened in regard to the pathogenesis of hyperlipemia in nephrosis no solution of this puzzling problem has been found.

POSSIBILITY OF A UNITARIAN CONCEPT OF DIFFERENT ETIOLOGICAL AGENTS

From available experimental and clinical data, the idiopathic nephrotic syndrome in man could be due to at least two different etiological mechanisms: a chemical agent or an antibody-antigen reaction. The first possibility is suggested by the experimental production of nephrosis in rats by the aminonucleoside derived from puromycin and by trimethadione and by the observation of a nephrotic renal disease in patients treated with trimethadione or penicillamine.[49] The possibility that these agents may produce

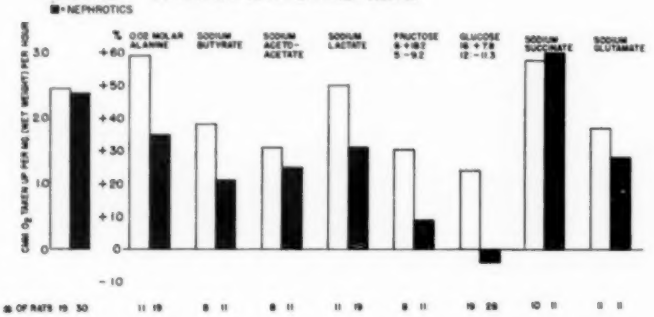

Fig. 5. Average values of oxygen uptake of renal cortex slices from control (white columns) and nephrotic rats (black columns). The first two columns represent observations without addition of substrates to Krebs-Ringer's solution. The next 8 double columns represent the per cent difference of oxygen uptake after addition of various substrates. Note the diminished increase in oxygen uptake in nephrotic slices after the addition of fructose and glucose.

the disease process by rendering usually nonantigenic kidney proteins antigenic has to be kept in mind even though experimental studies have thus far failed to support this theory.[7, 10] The second possibility of a primary immunochemical process assumes antibodies to certain kidney proteins and is suggested by experimental studies using heteronephrotoxic sera and by the demonstration that renal iso- and autoantibodies can be produced in rats and lead to development of a nephrotic syndrome.[16] Certain observations of the disease in humans are compatible with such a theory, but proof is lacking. The possibility that a common denominator might link these two different agents should also be considered. By means of the Warburg technique it has been shown that the renal cortex of rats injected with rabbit anti–rat kidney serum does not utilize glucose and fructose normally.[50] This metabolic lesion was noted within a day after the injection of nephrotoxic serum and persisted as long as did the induced renal disease (Fig. 5). It was observed for many months after the presence of heteronephrotoxic antibodies in renal tissue could no longer be assumed. The antibody-antigen junction thus induced a metabolic lesion that can become irreversible. Such a reaction could explain the chronicity of the disease after a single, brief injury. It would also provide a link between the chemical and immunochemical production of this syndrome if future studies should reveal an identical or similar metabolic alteration in the renal cortex of rats which had received aminonucleoside or trimethadione.

THE UNITARIAN CONCEPT OF THE NEPHROTIC SYNDROME IN MAN

The separation of "nephrosis" from "nephritis" was originally based on the assumption that the former represented a degenerative disease of the tubules, whereas the latter was caused by an inflammatory process within the glomeruli.[1, 2] We have discussed the evidence indicating that the primary site of the nephrotic renal process is in the glomerulus.[4, 5, 14, 25] The original classical hypothesis of Mueller[1] and Fahr[2] thus is no longer tenable. Nonetheless, the concept persists that the nephrotic patient who at no time has had hematuria, azotemia, or hypertension has a different disease entity than the one who has had hematuria, at times is hypertensive, has some azotemia, and who may later die in renal failure. The term "pure nephrosis" has been used to identify the former, and such terms as "mixed form of nephrosis," and "nephrosis with nephritis" for the latter.[51] That one and the same etiological agent, the heteronephrotoxic antibody, can produce these two supposedly different forms of disease depending on certain differences in the experimental conditions in the rats has supported a unitarian view. The age distribution at onset of these two forms of the nephrotic syndrome in children is identical and is different from that of acute and chronic glomerulonephritis.[51] The unitarian concept also explains why a nephrotic child may be entirely free of so-called "nephritic" symptoms on one admission and may later have hematuria and/or hypertension with or without azotemia, which in turn may disappear.[53] These transitions formerly required a change of diagnosis and prognosis. They are looked upon today as varying manifestations of a single disease process. The observation of so-called familial nephrosis,[54] where 1 of 4 children had the symptomatology of so called "pure nephrosis" while 3 other similarly affected siblings had symptoms of more severe glomerular damage, fits into the unitarian concept of this disorder.

The validity of this unitarian conception will remain debatable as long as the etiology of nephrosis remains unknown. The newer concept is, however, better adapted to clinical observations and has proved to be more accurate in relation to treatment and prognosis.

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The pathology of the nephrotic syndrome

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MINNEAPOLIS, MINN.

THE nephrotic syndrome is a clinical term useful in describing patients with signs and symptoms including edema, severe proteinuria, hypoproteinemia, and hypercholesterolemia. Other terms have been used to describe the various clinical forms of the syndrome and its relationship to a variety of pathologic lesions, e.g., nephrosis, pure nephrosis, lipoid nephrosis, and mixed nephritis-nephrosis. The difficulties of semantics are not likely to be solved until the specific etiological agents responsible for the various forms of the nephrotic syndrome are identified. In this discussion, the nephrotic syndrome which occurs in the absence of recognizable etiological agents or systemic disease will be designated the *idiopathic* nephrotic syndrome in an attempt to avoid confusion of terminology.

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The nephrotic syndrome may also occur in association with such systemic diseases as diabetes mellitus and lupus erythematosus; in the course of recognized renal disease such as chronic glomerulonephritis and renal vein thrombosis; and following exposure to a variety of toxins, drugs, and other agents. These *specific* forms of the nephrotic syndrome will be referred to as such in the present report.

The primary event leading to the development of the multiple clinical symptoms and signs encompassed by the term nephrotic syndrome is believed to be proteinuria. Although the concept of proteinuria as the basic pathophysiologic defect in the nephrotic syndrome might appear to be an oversimplification in the light of the many abnormalities of function which have been demonstrated to exist in the fully developed syndrome (as discussed in other sections of this symposium), the concept serves to focus attention on the kidney as the primary organ involved in the pathogenesis of the disease. It also emphasizes the basic renal lesion which is generally recognized to be increased permeability of the glomerulus to protein.

A review of the older literature regarding the pathology of the kidney in the idiopathic nephrotic syndrome reveals that a wide

range of morphologic changes have been observed in specimens from patients dying of the nephrotic syndrome. Glomerular morphology was frequently found to be normal in both children and adults in whom infection terminated the idiopathic nephrotic syndrome relatively early in the course of the disease. However, prominent thickening of the basement membranes has been generally regarded by pathologists as the most common glomerular alteration observed on microscopy of autopsy tissue from patients with the nephrotic syndrome, and this lesion is regarded by some as the characteristic renal morphologic abnormality of the syndrome. Still other patients, usually those with hypertension and renal insufficiency, show variable degrees of glomerular hypercellularity, basement membrane thickening, and fibrosis.

The advent of percutaneous renal biopsy has made it possible to study the early morphologic changes which occur in patients with the nephrotic syndrome and to evaluate the sequence of pathologic events throughout the course of the disease by means of serial biopsies in the same patient.

The purpose of this report is to present the results of a study of renal biopsy specimens from 54 children with the nephrotic syndrome. Included are 40 children with typical idiopathic nephrotic syndrome and 14 children with various specific forms of the nephrotic syndrome. Serial specimens of kidney from 16 of the patients are reviewed in order to demonstrate the progressive changes in morphology which occur and to evaluate the effects of adrenocortical steroid therapy on the pathology of the disease.

MICROSCOPIC GLOMERULAR PATHOLOGY IN NEPHROSIS

Microscopic slides of 73 renal biopsy specimens obtained from 54 children with the nephrotic syndrome were evaluated by two observers who had no knowledge of the source of the tissue. Each observer recorded a description of the morphologic changes, a pathologic diagnosis, and a prediction of the eventual outcome of each patient as suggested by the pathology of the individual biopsy specimen. The outcome predicted and the morphologic descriptions resulting from this "blind" reading of the slides were then correlated with the observed response to treatment and the present clinical status of each patient.

Initial renal biopsy specimens from the 40 children with a typical idiopathic nephrotic syndrome were evaluated with regard to:

1. The relationship of the morphologic changes in the kidney to the duration of the disease.
2. The relationship of renal pathology to the effects of adrenocortical steroid therapy.
3. The reliability of predictions of outcome based on the histologic appearance.

Initial renal biopsy specimens from 21 children with the nephrotic syndrome of less than 6 months' duration at the time of biopsy were compared with initial biopsy specimens from 19 children with disease of longer duration. The average age at onset of the disease was comparable in the two groups: 4.9 and 4.7 years, respectively (Table I). Average values for the frequency of each type of morphologic alteration were determined from the "blind" readings recorded by the two observers. The marking system used in Table I designates the relative prominence of the various types of glomerular abnormality encountered as graded from 1 to 4. Thus, 4 plus marks (++++) in the normal column indicate that all glomeruli in the specimen were considered normal, while 4 plus marks in the fibrosis column indicate hyalinization of all glomeruli. A single plus mark (+) in a given column indicates that the lesion was present in only an occasional glomerulus. A similar grading system was used to designate tubular atrophy. Biopsy specimens indicated as showing minimal changes of either hypercellularity or basement membrane thickening were often considered normal by one of the observers. The distinction between a normal specimen and a minimally abnormal specimen is obviously arbitrary. Further, it is readily admitted that such a grading system does not permit a clear, simultaneous demon-

Table I. Clinical and pathologic data: initial renal biopsy in 40 children with nephrosis

	Case	Age at onset (years)	Duration at biopsy (months)	Morphology of renal biopsy*					Response to treatment†	Present status‡	Duration since biopsy (months)
				Glomeruli				Tubular atrophy			
				Normal	Hypercellular	Membrane thickening	Fibrosis				
Early	1	2.5	1	++++					C	Well	20
	2	5.5	1	+	+	++			O	Alive	11
	3	3.0	6	++	+	+			C	Well	2
	4	3.5	5	++	+	+			C	Well	3
	5	3.5	2	++	+	+			C	Well	10
	6	2.5	2	++	+	+			C	Well	42
	7	3.5	1	+++		+			C	Well	41
	8	2.5	1	++	+	+			(–)	Well	41
	9	4.0	1	++	+	+			C	Well	38
	10	3.0	1	+	+	++			C	Well	44
	11	5.5	4		++	++			P	Well	42
	12	2.0	2	++	+	+			C	Well	19
	13	3.0	1	++	+	+			C	Well	55
	14	3.5	6	++	+	+			C	Well	48
	15	12.5	5	+	+	++			P	Alive	51
	16	5	2	++++					P	Well	57
	17	5.5	2	+	+	++			O	Dead	34
	18	12	3	+++	+				P	Alive	49
	19	10	2	+++	+				C	Dead	3
	20	10	2	+++	+				C	Dead	2
	21	3	4	+	+	++		+	O	Dead	31
	Average	4.9									33
Late	22	2	14		++	++			P	Well	46
	23	7	22	++	+	+		+	P	Well	6
	24	1.5	39		+	+	++	+	P	Alive	41
	25	3	36	++	+	+			C	Well	28
	26	2	60	+	++	+			C	Well	40
	27	11	66			++	++	++	(–)	Alive	37
	28	9	27			++	++	+++	O	Alive	21
	29	2	13	++	+	+			P	Well	29
	30	2	32	++	+	+			C	Well	46
	31	6	39			++	++		P	Alive	58
	32	1.5	22		+	++	+	++	(–)	Dead	3
	33	10	24			++	++	+++	O	Dead	43
	34	13	30		+	+	++	+++	O	Dead	6
	35	2.5	24	++++					R	Well	5
	36	2.5	36	+++	+				R	Well	41
	37	5.0	64	+++	+				R	Well	41
	38	1.5	19	+++		+			R	Well	18
	39	4	36	+++			+		R	Well	42
	40	4	22	++++					R	Well	42
	Average	4.7									33

*See text for explanation of grading system.

†O = no response; P = diuresis, but continuing proteinuria; C = complete remission; (-) = not treated; R = clinical remission at time of initial biopsy (See text for details of treatment).

‡Well = no abnormalities. Alive = continuing evidence of renal disease.

stration of both the frequency and the severity of the pathologic changes within the specimen.

Fourteen of the 21 early biopsy specimens (obtained within 6 months of onset) were considered to be either normal or minimally abnormal (Fig. 1*A*). The other 7 demonstrated more definite hypercellularity and/or basement membrane thickening (Fig. 1*B*). None showed severe fibrosis or tubular atrophy.

Nine of the 19 late specimens (obtained more than 6 months after onset) demonstrated minimal abnormalities similar to those described above. Six of the 9 biopsies were obtained from patients in whom remission had been induced by adrenocortical steroid therapy prior to the initial biopsy. The other 10 specimens showed more severe membranous and proliferative glomerular lesions and frequently showed glomerular fibrosis and tubular atrophy.

The difference in the severity of the microscopic abnormalities between the biopsies obtained early and late in the course of the disease suggests that glomeruli are damaged progressively with increasing duration of the nephrotic syndrome, even in those patients who subsequently recover. However, the occasional patient with disease of long duration who showed only minimal morphologic abnormalities at the time of renal biopsy indicates that progression is not inevitable.

The increasing severity of the renal glomerular lesions with continuing disease is more convincingly demonstrated by the serial renal biopsy specimens in patients who failed to respond to therapy. Four patients showed minimal abnormalities in the initial biopsy and, following repeated therapeutic failures, showed severe glomerular damage in the subsequent biopsy. Fig. 2 illustrates the clinical course of such a patient. Despite early, repeated treatment, this child continued to manifest all of the signs of the nephrotic syndrome and within a year had clinical evidence of renal insufficiency, including severe depression of the creatinine clearance. Fig. 3*A* shows typical glomeruli from a renal biopsy obtained early in the course of the disease, and Fig. 3*B* illustrates a typical microscopic field from the biopsy specimen obtained two years later. We have previously reported similar progression of renal pathology from normal glomeruli to diffuse glomerular scarring in children with familial nephrosis.[1]

Adrenocortical steroid therapy resulted in complete remission of the nephrotic syndrome in the majority of patients whose renal biopsies prior to treatment showed minimal glomerular abnormalities. The response recorded (Table I) refers to the treatment given immediately following the renal biopsy in each patient. Treatment consisted of 14 or more days (usually 21 to 28 days) of cortisone in a dose of 300 mg. per square meter of body surface area or an equivalent amount of another adrenocortical steroid. In addition, nitrogen mustard was often given to those patients who had previously demonstrated "resistance" to adrenocortical steroid therapy.[2]

Satisfactory response to steroid therapy was much less common in patients whose renal biopsy specimens showed more severe glomerular lesions. Thus, of the 13 treated patients with few or no normal glomeruli (Table I), only 2 had complete remissions and 6 failed to respond at all to treatment. On the other hand, 14 of the 18 treated patients who had more than 50 per cent normal glomeruli had complete remissions, and none failed to respond. However, exceptions to these general observations were encountered. Two patients (Cases 11 and 15), whose renal biopsies showed moderately severe membranous lesions, ultimately improved after repeated treatment with adrenocortical steroids and are clinically well and in partial laboratory remission more than 3 years after treatment. Three patients (Cases 2, 17, and 21) failed to respond to treatment, in spite of the fact that the abnormalities noted in renal biopsy specimens were relatively mild.

Patients in whom a repeat biopsy was done following successful adrenocortical steroid therapy demonstrated an improvement in the histologic appearance which paralleled the clinical improvement. Figs. 4*A* and 4*B* illustrate the response of a patient with a mild proliferative lesion whose repeat biopsy following cortisone therapy shows normal glomeruli.

Serial renal biopsies also suggest that successful steroid therapy may prevent progression of glomerular damage over a number of years of recurrent episodes of the nephrotic syndrome. Fig. 5 illustrates a representative glomerulus from a second renal biopsy speci-

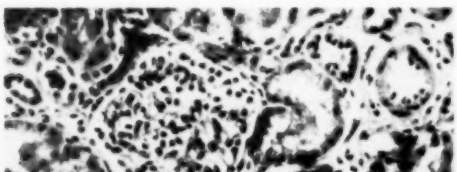


Fig. 1*A*. Case 1. Two representative glomeruli with mild hypercellularity and slight thickening of the basement membrane in a few capillaries. (Hematoxylin and eosin stain. Original magnification ×300.)

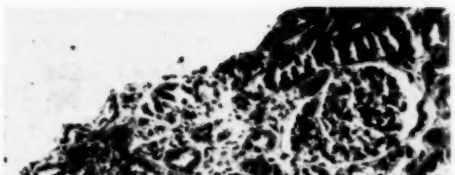


Fig. 1*B*. Case 11. Moderate hypercellularity and more marked thickening of the basement membranes are present. The glomerulus on the left shows early fibrosis. (Hematoxylin and eosin stain. Original magnification ×300.)

men from a patient with disease of 3 years' duration (Case 6). During this period, the disease recurred 5 times but responded completely to cortisone therapy each time. Despite the duration of the disease and the frequent exacerbations, his last biopsy showed less abnormality than his original biopsy.

Normal kidney biopsy specimens were also obtained from 6 patients (Cases 35 to 40, Table I) during remission and after prolonged and recurrent episodes of the nephrotic syndrome (duration 19 to 64 months). The clinical course (Fig. 6) and renal morphology (Fig. 7) of a typical patient from this group are presented. The renal biopsies in these patients suggest that successful adrenocortical steroid therapy of multiple exacerbations of the disease may play a role in preventing the progressive renal damage which may otherwise occur in the nephrotic syndrome.

Thus, our observations on the effect of adrenocortical steroid therapy on the pathology of typical idiopathic nephrotic syndrome support the following hypotheses: (1) Response to treatment more often occurs in patients with minimal renal pathology. (2) Successful therapy results in a decrease in the severity of the glomerular abnormalities. (3) Treatment may prevent otherwise progressive renal damage in patients with recurrent disease. In support of the first of these hypotheses, McCrory and colleagues[3] have reported a positive correlation between the severity of renal functional abnormalities and the response to adrenocortical steroid therapy. Galán and Masó[4] have confirmed the second hypothesis in a demonstration of improvement in the morphology of renal biopsy specimens from patients responding to adrenocortical steroid therapy. The third hypothesis will require additional studies for confirmation since there is no way to determine that a patient's disease would have progressed if he had not been treated.

Earlier studies of renal biopsy specimens from children with the nephrotic syndrome, reported from our laboratories,[5] suggested that the outcome of the disease might be related to the severity of the microscopic pathology. If confirmed, this hypothesis would appear to have practical value for the physician employing renal biopsy as an aid in the management of patients with nephrotic syndrome. The need for better prognostic criteria is emphasized by the fact that clinical signs and measurements of renal function have not proved to be reliable guides to prognosis in a given child.[6]

In the present study, the probable outcome of 40 children with idiopathic nephrotic syndrome was predicted by two observers. Pre-

dictions were based upon the observers' impressions of the over-all severity of the microscopic pathology in renal biopsy specimens. Both the degree of damage observed in individual glomeruli and the frequency of occurrence of significant lesions in the total specimen were considered in estimating the probable prognosis.

The results of the study show agreement between the predicted outcome and the observed outcome of the disease in 33 of the 40 cases. The observed outcome is necessarily tentative since the children have not been followed long enough after the initial renal biopsy (average 33 months) to be sure that the final outcome of the disease is known. Five of the 7 patients in whom a poor correlation between the severity of the morphologic lesion and the observed outcome was seen have been discussed previously (Cases 2, 11, 15, 17, and 21). The other 2 patients (Cases 19 and 20) died of causes unrelated to renal failure, one of infection and the other of carotid artery thrombosis.

It seems apparent that, although there was general agreement between the severity of the renal damage in the initial biopsy specimen and the observed outcome of the children with the idiopathic nephrotic syndrome in this series, exceptions to the general rule occurred with sufficient frequency to make clinical application of the rule to any one patient of doubtful value.

Renal biopsy specimens from 14 children with specific forms of the nephrotic syndrome (including familial nephrosis, infantile nephrosis, and the nephrotic syndrome following mercury poisoning, bee stings, and diabetes mellitus) were reviewed in order to compare the pathology of these forms of the disease with that of the idiopathic nephrotic syndrome. We have pointed out in a previous study[1] that a familial form of the nephrotic syndrome, occurring in 4 children in one family, was not distinguishable from the idiopathic nephrotic syndrome on the basis of the microscopic pathologic changes. Subsequent renal biopsy specimens from 2 of the 3 children surviving at the time of that report showed progressively severe microscopic renal glomerular changes with continuing disease, and both of these children have died with renal insufficiency. A child from another family in which 2 children developed the nephrotic syndrome early in life is also included in the present study. A renal biopsy early in the course of the disease showed minimal proliferative glomerulitis, and an autopsy 3 years later demonstrated hyalinization of most of the glomeruli from both kidneys.

Microscopy of a renal biopsy specimen from a child with the nephrotic syndrome

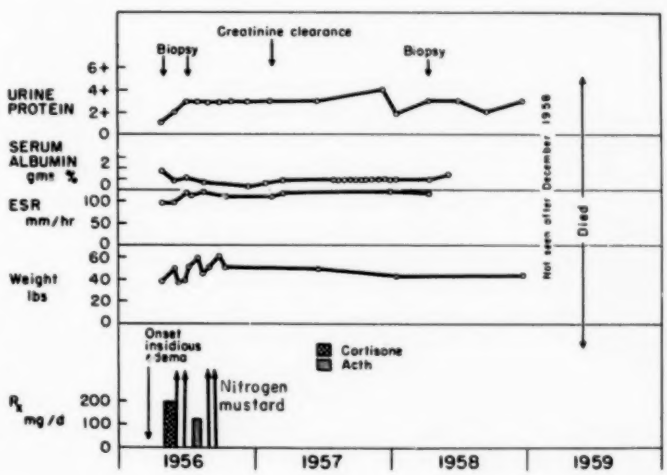


Fig. 2. The course of nephrotic syndrome in Case 17 demonstrating refractoriness to therapy, gradual deterioration of renal function, and death in 36 months.

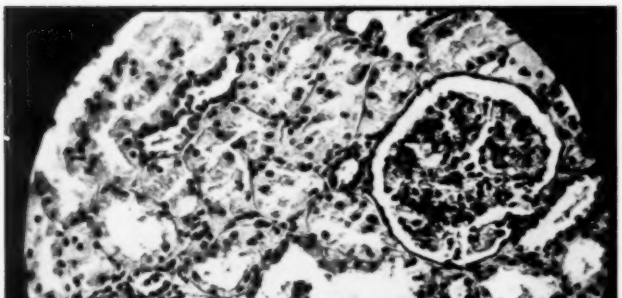


Fig. 3*A*. Case 17. Initial biopsy, showing relatively mild proliferative and membranous changes. (Hematoxylin and eosin stain. Original magnification ×300.)

which followed exposure to and absorption of mercury showed minimal glomerular hypercellularity and focal capillary membrane thickening. This abnormality was apparently reversible since the child recovered completely and has remained well for 3 years.

Renal biopsy specimens from 2 children in whom the nephrotic syndrome followed bee stings revealed glomerular hypercellularity and focal capillary membrane thickening in one case, and moderately severe membrane thickening and glomerular hyalinization in the other case. The latter patient, whose renal biopsy showed moderately severe fibrosis of more than half of the 50 glomeruli in the specimen, has recovered completely after treatment with malaria and is well 4 years after the biopsy. The recovery of this patient is remarkable, and it is difficult to reconcile the disparity between the morphologic and clinical features of this patient with our total experience.

A renal biopsy specimen from a child who developed the nephrotic syndrome 4 years after the onset of diabetes mellitus showed glomerular hypercellularity and minimal focal thickening of the glomerular capillary membranes. Nodular or linear hyaline masses, typical of the renal disease of diabetes, were not found.

We have previously reported a description of the pathologic changes observed in 12 infants with the nephrotic syndrome (infantile nephrosis).[8] The striking tubular dilatation found in renal biopsy specimens and autopsy specimens from 4 of these infants was the unique morphologic abnormality observed. The other 8 patients with infantile nephrosis showed a spectrum of glomerular abnormalities indistinguishable from those seen in patients with typical idiopathic nephrotic syndrome.

The extreme resistance to therapy of patients with familial and infantile forms of the nephrotic syndrome does not appear to be related to the severity of the microscopic renal pathology.[1, 7-9] To the best of our knowledge, none of the patients with either the infantile or the congenital-familial disease has responded to treatment with adrenocortical steroid therapy. Predictions of the outcome of either of these forms of the nephrotic syndrome, based solely on the abnormalities of microscopic structure, are thus highly unreliable.

THE ULTRAMICROSCOPIC PATHOLOGY OF NEPHROTIC SYNDROME

The earliest investigations of the renal pathology of the nephrotic syndrome, using electron microscopy, demonstrated a consistent abnormality of the glomerular capillaries in each of 4 children from one family.[10] The abnormality consisted of fusion of the foot processes of the epithelial cell into a continuous mass of epithelial cytoplasm about the external surface of the basement membrane. Epithelial cell foot processes were uniformly either entirely lacking or greatly distorted in all the capillaries of each glomerulus studied. The nephrotic syndrome in each of the 4 children in this family was clinically indistinguishable from the idiopathic nephrotic syndrome at the onset of the disease, but at the time renal biopsies were obtained 2 of the children had clinical signs of hypertension and renal insufficiency. The child who had normal renal function at the time of biopsy was of special interest since light microscopy revealed no glomerular abnormalities, but electron microscopy showed a severe epithelial cell foot process abnormality. In addition to the epithelial cell lesion, glomeruli from the children in whom the disease had progressed clinically to some degree of renal failure, or in whom hypertension had developed, showed endothelial cell proliferation, basement membrane thickening, and deposits of basement membrane material between endothelial cells.

These original observations have been confirmed in our laboratories by subsequent study of renal biopsies from many children and adults with the nephrotic syndrome of diverse etiology and circumstance (Figs. 9 and 10).[11-13] Similar abnormalities of the glomerular epithelial cell foot processes have been demonstrated by other investigators in reports describing the nephrotic syndrome in children[14, 15] and adults.[14, 16] Although a variety of associated changes in the glomerular endothelial and basement membrane morphology has been observed in studies of renal tissue from patients with the nephrotic syndrome, distortion of the epithelial cell foot processes has been the most consistent abnormality found in all cases reported to date.

The epithelial cell foot process lesion observed in children with the nephrotic syndrome is often the only definite abnormality

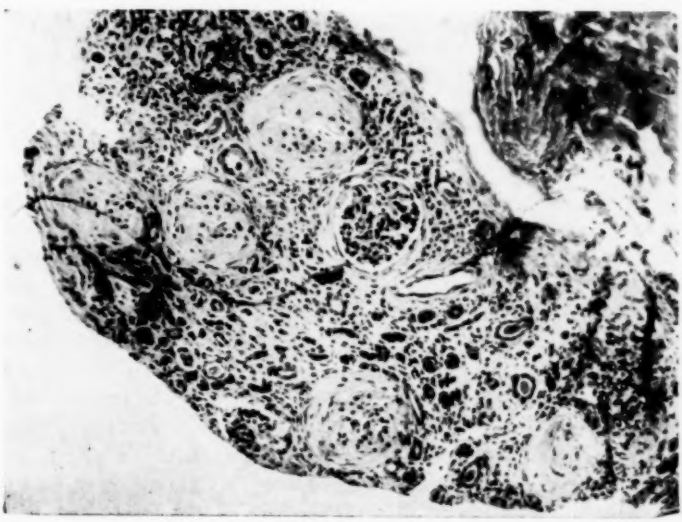


Fig. 3*B*. Case 17. Final biopsy, showing severe hyalinization of most of the glomeruli and severe tubular atrophy. (Hematoxylin and eosin stain. Original magnification ×300.)

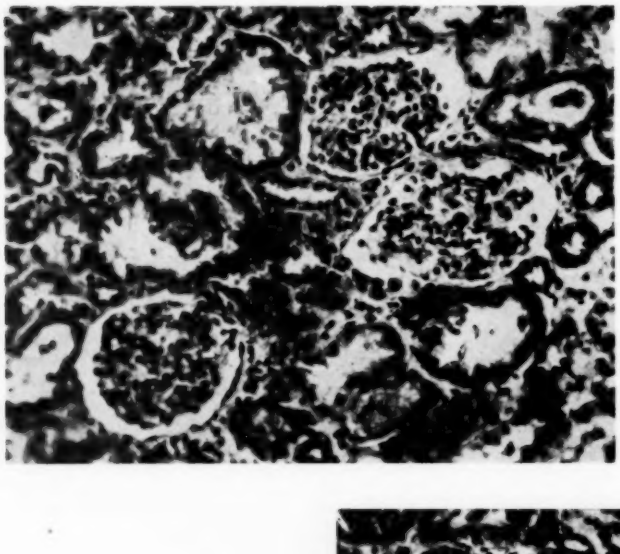


Fig. 4*A*. Case 22. Initial biopsy prior to therapy. Mild hypercellularity and questionable basement-membrane thickening are present. (Hematoxylin and eosin stain. Original magnification ×270.)

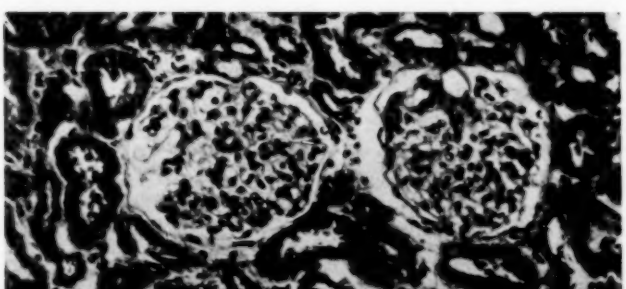


Fig. 4*B*. Case 22. Subsequent biopsy 6 months later, in remission, showing decreased cellularity and relatively normal basement membranes. (Hematoxylin and eosin stain. Original magnification ×300.)

of ultrastructure, and the glomeruli may appear to be normal when studied by light microscopy. The basement membrane (lamina densa) is usually normal in width and homogeneous in density, and the endothelium is often entirely normal. However, electron microscopy of glomeruli from children with the nephrotic syndrome of longer duration (greater than 6 months) usually reveals abnormalities of both the endothelium and the basement membrane. The early endothelial lesions consist of broadening (swelling) of the endothelial cytoplasm, formation of blebs of endothelium which project into the capillary lumen, and accumulation of dense basement-membrane material beneath the endothelial membrane and between endothelial cells (Fig. 11). Accumulation of dense basement-membrane material between the normal basement membrane and the endothelium contributes to the appearance of thickening of the "basement membrane," and occasionally no clear demarcation between the original (true) basement membrane and the apparently newly formed material can be discerned. Basement-membrane thickening may thus be due in part to a contribution of material with

the morphologic and tinctorial properties of lamina densa on the endothelial side of the original basement membrane. There is reason to believe that the material may be derived from or be produced by the endothelial cells.[11, 13]

Farquhar,[17] Fiaschi and associates,[14] and Movat and McGregor[18] have observed that the thickening of basement membrane which occurs in adults with the nephrotic syndrome differs from that seen in children. Whereas the basement membrane thickening which occurs in children with the nephrotic syndrome is diffuse and symmetrical and appears to proceed from the endothelial side, the thickening of the basement membrane seen in some adults with the disease is very irregular in contour and appears to arise from the epithelial side of the original basement membrane. The accumulated basement-membrane material on the epithelial side of the original membrane is described by Farquhar[17] as less dense than normal early in the process of basement-membrane thickening. In more advanced lesions, the newly formed basement-membrane material may appear to incorporate the damaged epithelial cell foot process layer and become much more dense than the original basement membrane.

The nephrotic syndrome which occurs in association with the renal disease of diabetes and lupus erythematosus[19] is also associated with a loss of normal epithelial cell foot processes. In these diseases, the epithelial cell lesion occurs in association with prominent abnormalities of the lamina densa. Renal amyloidosis, when associated with the nephrotic syndrome, reveals a similar epithelial cell abnormality.[17]

The nephrotic syndrome occasionally occurs as a result of Tridione medication, following bee stings, and as a result of mercury ingestion. Electron microscopic studies in our laboratories have shown that these forms of the nephrotic syndrome also demonstrate a severe epithelial cell foot process lesion as the principal abnormality. A single case of renal vein thrombosis which resulted in the nephrotic syndrome has been studied by electron microscopy,[20] and coalescence of the epithelial cell foot processes was the only microscopic abnormality noted in this patient. Finally, in the course of a study of a number of infants with the nephrotic syndrome, 4 of whom were shown to have an unusual form of "cystic" tubular abnormality, electron microscopy of renal biopsies revealed fusion of the epithelial cell foot processes as the prominent glomerular lesion.[8]

Three types of experimental nephrotic syndrome—nephrotoxic serum disease in rats[21] and mice,[22] iron oxide nephrosis in rabbits,[23] and aminonucleoside nephrosis in rats[24, 25]—have been studied by electron microscopy. These experimental forms of nephrosis have all been shown to be associated with an obvious epithelial cell foot process abnormality. Further, the development of the foot process lesion in aminonucleoside nephrosis has been shown to coincide with the appearance of proteinuria in the rat.

Thus, virtually all forms of renal disease which are characterized by proteinuria, both those occurring in man and those experimentally induced in laboratory animals, are associated with abnormal glomerular epithelial cell foot processes. An additional important morphologic correlation between proteinuria and the epithelial cell foot process lesion is provided in the observation

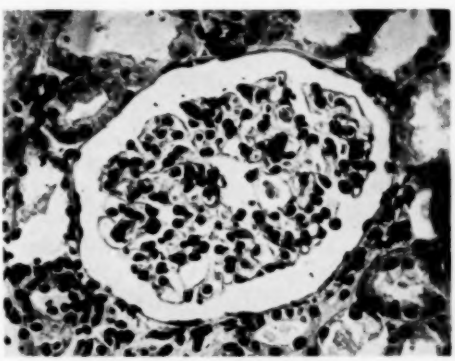


Fig. 5. Case 6. A representative glomerulus in a kidney biopsy obtained three years after the onset of nephrotic syndrome. The only evidence of abnormality is slight hypercellularity. (Hematoxylin and eosin. Original magnification ×500.

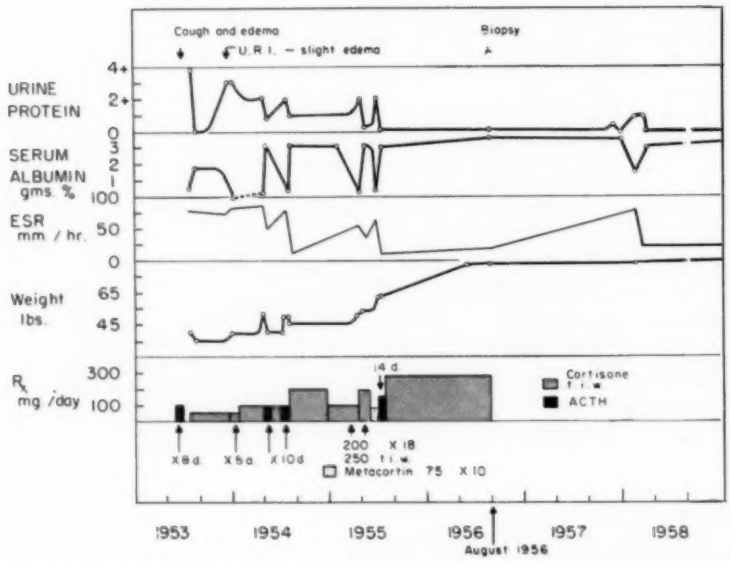


Fig. 6. Case 39. Course, showing frequent exacerbations during the first 2 years of therapy and complete remission during the next year. The biopsy was obtained just before the end of the intermittent cortisone therapy.

from our laboratory[5, 13] and by Folli and co-workers[16] of reversibility of the foot-process abnormality by successful treatment of the nephrotic syndrome in children and adults. Further, our studies of aminonucleoside nephrosis[24] show that the epithelial cell foot processes in glomeruli of rats which recover from the disease return to normal with cessation of proteinuria.

We have previously commented that the epithelial cell foot processes are less regularly distorted in glomeruli which show fibrosis than in those which appear normal by light microscopy. It is apparent from our studies that the extent of foot-process abnormality is related to the severity of proteinuria. Renal biopsy specimens from children in partial remission (1+ proteinuria) (Fig. 12) show a "spotty" foot process lesion, whereas specimens from children in complete remission (negative urine protein test) show normal epithelial cell foot processes (Fig. 13).

Fiaschi and co-workers[14] have commented, and we have observed, that minor degrees of epithelial cell foot process fusion occur in patients with other renal diseases in which mild proteinuria is present in the absence of edema or hypoproteinemia (chronic glomerulonephritis and chronic pyelonephritis). However, the changes in basement-membrane thickness and the endothelial proliferation, which regularly are visible at the microscopic or submicroscopic level in these other diseases, are more prominent than are the "spotty" foot-process abnormalities. Characteristically, the basement membrane (lamina densa) is of normal width early in the course of the nephrotic syndrome (2,800 A.U. or less),[26] whereas some degree of basement-membrane thickening is usually present in the nephrotic syndrome of longer duration and in chronic glomerulonephritis, lupus erythematosus,[11] and diabetic nephropathy.[19]

Since fusion of the glomerular epithelial cell foot processes is present in all forms of severe proteinuria which have been studied and appears to be related to the amount of proteinuria, and since both the lesion and the proteinuria are reversible by treatment, two possible explanations of the relationship between the two phenomena might be sug-

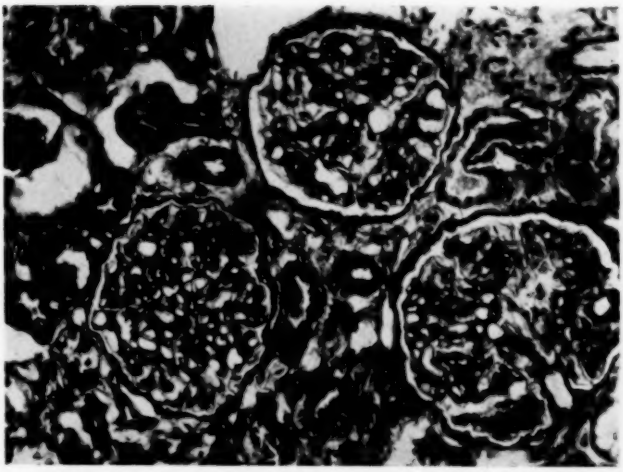


Fig. 7. Case 39. Minimal basement-membrane thickening, apparent with periodic acid–Schiff stain, but not obvious with hematoxylin and eosin stains, was the most severe abnormality observed in the group of patients in remission. (Periodic acid–Schiff stain. Original magnification ×320.)

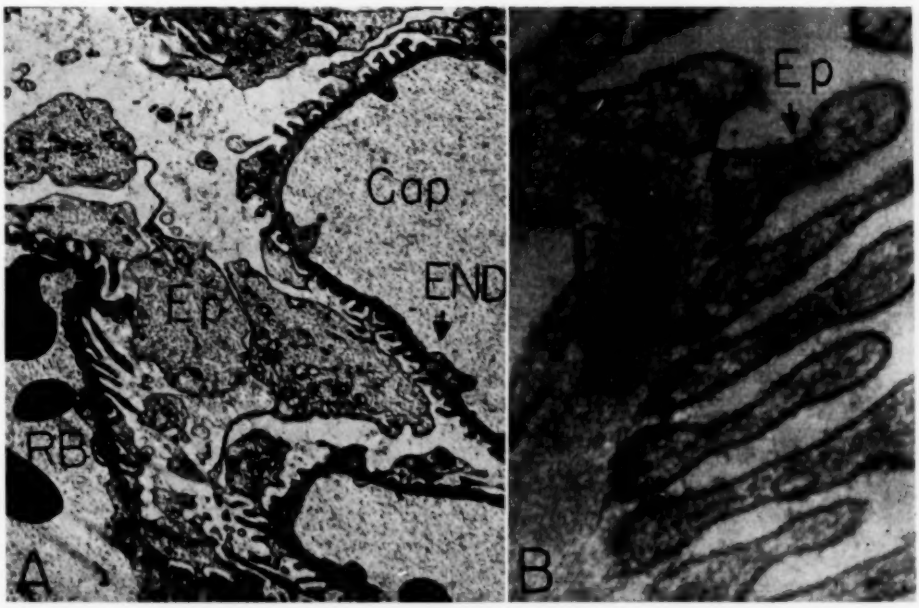
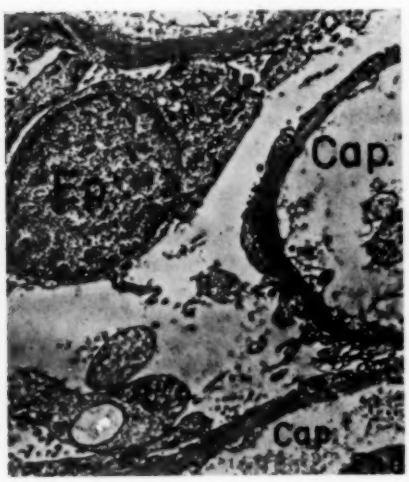


Fig. 8, *A* and *B*.* Electron micrographs of capillary membranes of a normal glomerulus. Cytoplasmic projections of the epithelial cells terminate as foot processes (arrow) at the lamina densa (basement membrane). The endothelium is very thin in most areas, and pores are visible at higher magnification, *B*. The basement membrane is homogeneous. (*A*, ×7,500; *B*, ×85,000.)

*Abbreviations for electron micrographs Figs. 8 through 13:
Ep = epithelial cell
End = endothelial cell
BM = basement membrane (lamina densa)
RBC = red blood cell
Cap = capillary lumen



A

B

Fig. 9, *A* and *B*. A portion of a glomerular capillary from a child with a nephrotic syndrome of one month's duration is illustrated. The epithelial border of the basement membrane is covered by a layer of cytoplasmic material, and normal foot processes are absent. The basement membrane is normal. *B*, A higher magnification view of the same capillary wall is shown. (*A*, ×5,200; *B*, ×19,800.)

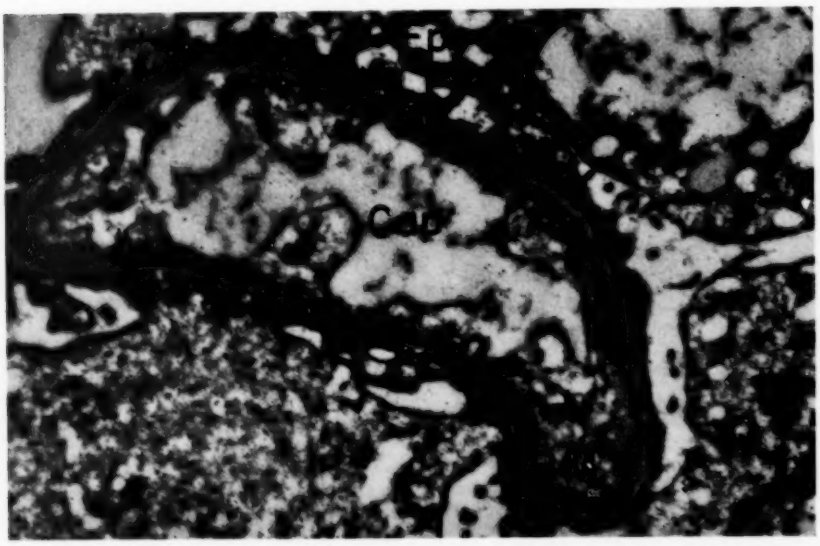


Fig. 10. Typical capillary loops in a renal biopsy specimen from a 52-year-old man with an idiopathic nephrotic syndrome are shown. Small blebs of endothelial cytoplasm protrude into the lumen of the capillary. The basement membrane is minimally thickened. A large vacuole is present in the epithelial cell to the upper right. No normal epithelial cell foot processes are present. (×14,000.)

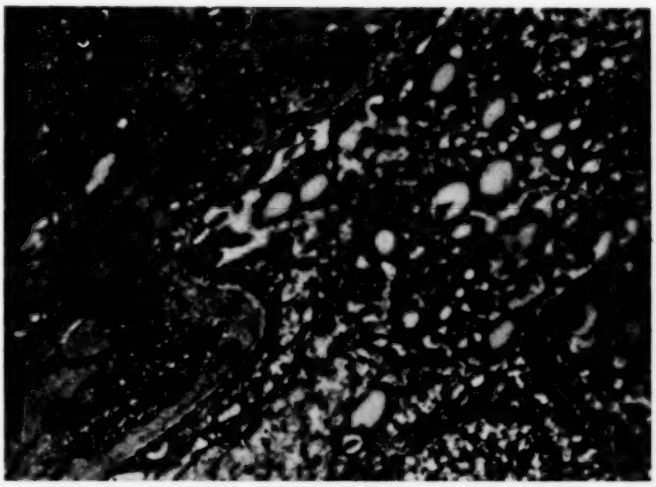


Fig. 11. Portion of a glomerular capillary wall in a child with a nephrotic syndrome of 2 years' duration. The basement membrane is greatly thickened by accumulation of material which is contiguous with the original basement membrane. The lumen to the left is occupied by endothelial cell cytoplasm and basement membranelike material. The typical epithelial changes, loss of foot processes, vacuole formation, and protein droplet accumulation, are present in the epithelial cell. (×11,000.)

gested.[10] It is possible that the epithelial cell is the site of the initial injury to the renal glomerulus and that injury of the epithelial cell, expressed as coalescence of the foot processes, results in proteinuria. On the other hand, the foot-process lesion might be considered to be the result of proteinuria rather than the cause. Hall[27] has suggested that the space between adjacent foot processes may act as a "filtration slit," and that changes in the size of the filtration slit might serve to regulate filtration of larger molecules (such as proteins). Fusion of the foot processes might then be interpreted as a response of the epithelial cells to proteinuria, an attempt, in the teleologic sense, to prevent proteinuria. In two recent studies, Farquhar and Palade[28] and Vernier and co-workers[29] have demonstrated increased pinocytosis of tracer molecules by the epithelial cells of nephrotic rats, as compared to that of control animals. The studies appear to support the concept that the basic defect lies within the basement membrane which is abnormally permeable to protein. Spiro[30] has described large holes or pores within the basement membrane of glomeruli from patients with the nephrotic syndrome which he considers to be visible at high magnification in renal tissue which has been stained with phosphotungstic acid. Using similar methods of preparation, we have not been able to reproduce this finding. We have previously reported the observation of rather large areas of low density which are occasionally seen in the basement membrane of renal glomeruli from patients with moderately severe basement-membrane thickening. These zones of low density sometimes give the basement membrane a "moth-eaten" appearance.[11] It should be pointed out that relatively few "leaks" of small size could easily explain the massive proteinuria characteristic of the disease, and it is entirely possible that pores which exist might escape detection despite a diligent search. The thinness of the sections used in electron microscopy and the random samples studied might easily account for the fact that pores which would permit albuminuria, yet not permit globulinuria, have not been described.

Further studies of the physiologic significance of the epithelial cell foot process lesion are in progress. This abnormality remains a unifying lesion of the renal glomerulus which appears to link the physiology and the pathology of the various forms of severe proteinuria.

DISCUSSION

Several recent reports of the study of renal biopsy specimens from adults with the nephrotic syndrome by light microscopy[31-33] described thickening of the glomerular basement membrane (membranous glomerulonephritis) as the most common abnormality encountered. Kark and co-authors[31] found this lesion to be present in 40 per cent of 98 specimens from adults, while minimal or no abnormalities were observed in 11 per cent of the specimens. In the present study, 66 per cent of the biopsy specimens obtained within 6 months of the onset of the idiopathic nephrotic syndrome in children were either normal or showed minimal abnormalities. The higher incidence of more severe glomerular abnormalities in adults may be related to such factors as longer duration of disease prior to biopsy, a different etiological mechanism or agent, or to a difference in the response of adult glomeruli to injury. With regard to the latter possibility, Bell[34] has pointed out that glomeruli from normal adults are relatively more cellular than glomeruli from normal children. It is possible that this difference in morphology or some other unrecognized difference in structure of the adult glomerulus is related to the increased frequency of membranous glomerular abnormality in that age group.

The data available from study of both autopsy and renal biopsy specimens from children and adults with the nephrotic syndrome suggest that the following sequence

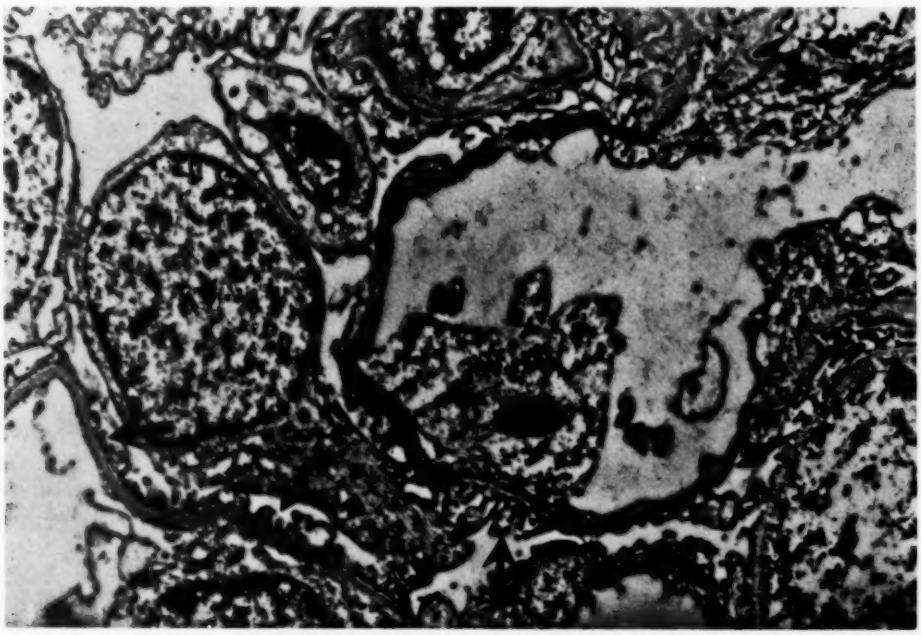


Fig. 12. A portion of a glomerulus from the kidney of a child with nephrotic syndrome in partial clinical remission. Diuresis and decreased proteinuria followed treatment with adrenocortical steroids. A few relatively normal epithelial cell foot processes are indicated by arrows. (×7,250.)

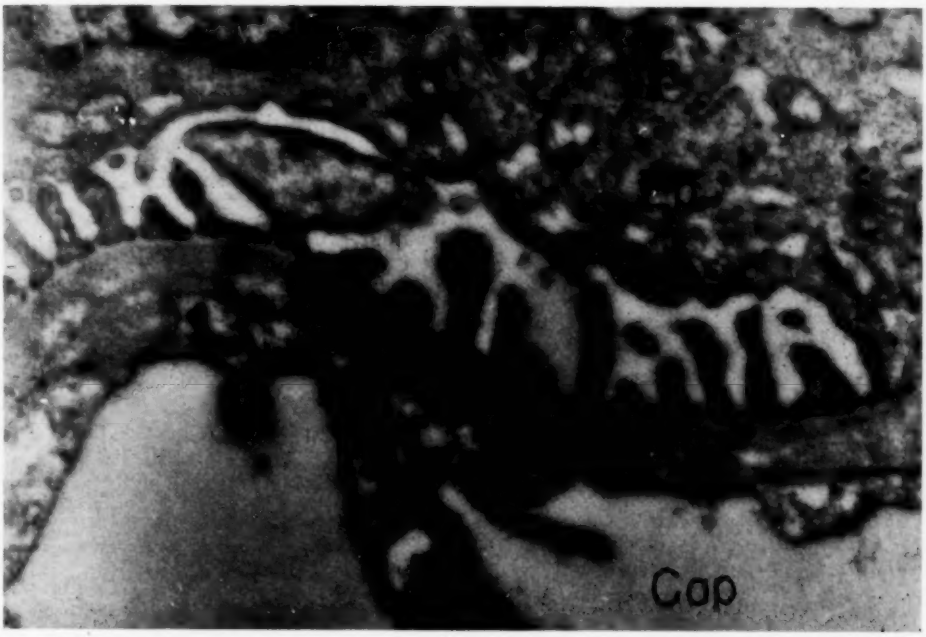


Fig. 13. An electron micrograph of a glomerular capillary from a renal biopsy obtained during complete clinical remission of nephrotic syndrome in a 4-year-old child. There are no apparent abnormalities. (×32,000.)

of morphologic events occurs in the glomerulus.*

The initial morphologic abnormality is fusion of the epithelial cell foot processes; it is revealed by electron microscopy. At this stage in the disease, no abnormalities may be apparent in the light microscope, even with the aid of special staining methods. Somewhat later in the course of the nephrotic syndrome, the glomerulus is usually hypercellular, and thickening of the basement membrane becomes evident by light microscopy. It has been pointed out[5, 11, 17] that the "basement membrane," as seen with the light microscope, includes the three layers of the glomerular capillary wall which may be resolved with the electron microscope. Thus, broadening of any one of the three layers, endothelium, lamina densa, or the epithelial cell foot processes, might be visualized as thickening of the "basement membrane" by light microscopy. Since the fusion of the submicroscopic processes also regularly involves an apparent condensation of the fused foot process cytoplasm, making it appear more compact in the nephrotic than in the normal, it seems likely that the earliest thickening of the capillary wall detected by light microscopy is due to the change in the epithelial cell foot processes.

Light microscopy of renal glomeruli from patients with the nephrotic syndrome of 6 months' duration or longer usually reveals a variable degree of endothelial cell proliferation and "basement-membrane" thickening. Electron microscopy of the same specimen confirms the presence of endothelial hypercellularity and demonstrates accumulations of basement-membrane-like material adjacent to the original basement membrane

*It is accepted that the generalizations which are made do not necessarily apply to all cases of the nephrotic syndrome. It is apparent from our studies that the severity of the disease and the rate of progression of the morphologic alterations may vary greatly from patient to patient. Occasionally, progression is rapid and proceeds in spite of "adequate" therapy.

and between endothelial cells. In this circumstance, both the newly formed basement-membrane material and the fused epithelial cell foot processes contribute to the appearance of thickening of the capillary wall as seen by light microscopy.

Electron microscopy of glomeruli from adults with the nephrotic syndrome may show additional abnormalities of the capillary wall. The electron micrographs of Fiaschi and colleagues[14] support the studies of Jones[35] who has shown splitting of the capillary wall by light microscopy, using sections stained with the silver-methenamine method.

The further progression of glomerular damage results in collapse of adjacent capillary loops and occlusion of the lumina of capillaries by hyaline material (lobular glomerulonephritis). Correlation of thin sections studied by phase microscopy or light microscopy, using a number of special staining techniques,[36, 37] with adjacent ultrathin sections studied by electron microscopy, has been helpful in demonstrating the nature of the hyaline material. These studies show that the dense, osmophilic basement-membrane material which accumulates in the late stages of the nephrotic syndrome and contributes to the processes of thickening of the capillary wall and glomerular fibrosis is identical with the hyalin of light microscopy.

The similarity of microscopic appearance of fibrotic glomeruli from patients dying of idiopathic nephrotic syndrome (nephrosis) and glomerulonephritis has led to the concept, held by some pathologists, that nephrosis and glomerulonephritis are the same disease. We have reviewed in this report the evidence that the early morphologic changes in nephrosis are limited to the epithelial cell. Previous reports from our laboratory[5, 11] have emphasized that the predominant abnormalities noted by electron microscopy of renal biopsies from patients with glomerulonephritis, both acute and chronic, involve the glomerular endothelial cells. These morphologic observations and the many differences in clinical expression in the two diseases would appear to support the concept that nephrosis and glomerulonephritis are indeed different diseases. The occurrence of the nephrotic syndrome in a patient with chronic glomerulonephritis is, in our opinion, the result of a secondary physiologic and morphologic abnormality superimposed on the underlying endothelial cell disease.

The pathogenesis of the nephrotic syndrome associated with amyloidosis, diabetes mellitus, and lupus erythematosus is more difficult to understand. It is possible that deposition of fibrinoid material within and beneath the basement membrane in the renal disease of diabetes and in lupus erythematosus[17] may alter in some way the permeability of the glomerular capillary wall to protein. Miller and Böhle[38] have suggested that the proteinuria of experimental amyloidosis in mice may be related to the infiltration of the basement membrane by porous amyloid material. Our electron microscopic studies of the nephrotic syndrome associated with diabetes mellitus, as well as the studies of Farquhar, Hopper, and Moon,[19] do not support Allen's[39] concept that the nephrotic syndrome develops in diabetic nephropathy because of thinning of glomerular capillary walls about nodular hyaline masses in the Kimmelstiel-Wilson syndrome.

We believe that important clues to an improved understanding of the pathogenesis of the nephrotic syndrome may be made available through further study of the experimental disease induced in rats by aminonucleoside, through a combination of approaches including biochemistry, histochemistry, and morphology, as recently proposed by Hartman.[40]

SUMMARY

The observations reviewed appear to support the following concepts:

1. Typical idiopathic nephrotic syndrome (nephrosis) of childhood and the other forms of the nephrotic syndrome discussed in this report are not morphologically distinguishable from one another, except for those in-

fants showing unusual renal tubular dilatation.

2. Progressive morphologic renal abnormalities occur during the clinical expression of the nephrotic syndrome over many months and years.

3. The severity of the microscopic pathology in patients who fail to respond to treatment is related to the duration of the disease. The rate of progression of the pathology is highly variable from patient to patient, and the factors which determine the rate of progression in an individual patient are unknown.

4. Patients with idiopathic nephrotic syndrome (nephrosis) in whom renal biopsy specimens reveal minimal glomerular abnormalities usually respond to treatment with adrenocortical steroids.

5. Cessation of proteinuria following adrenocortical steroid therapy is accompanied by improvement in the morphology of renal biopsy specimens.

6. Response to therapy with adrenocortical steroid hormones is usually associated with preservation of normal microscopic renal morphology, even though the nephrotic syndrome recurs for a number of years.

7. Patients in whom renal biopsies show more severe glomerular pathology often fail to respond to treatment with adrenocortical steroids. Morphologic alterations in the kidney are not, however, the only factors explaining the refractory state, since some patients with minimal lesions fail to respond to treatment, while a few patients with moderately severe pathology have obtained clinical remissions after therapy.

8. Predictions of the outcome of the idiopathic nephrotic syndrome, based on the severity of the morphologic changes in a renal biopsy, are generally reliable, but exceptions occur frequently enough to make the application of this method of prediction of doubtful value in individual cases. Such predictions are not valid in patients with either the familial or infantile forms of the nephrotic syndrome.

9. Electron microscopy of renal glomeruli reveals an abnormality of structure of the epithelial cell foot processes in all forms of the nephrotic syndrome which have been studied.

10. Similar abnormalities have been demonstrated in several forms of experimental nephrotic syndrome produced in laboratory animals.

11. The severity of the epithelial cell foot process lesion appears to be related to the severity of the proteinuria. The epithelial cell foot process abnormalities are also reversible, and normal structure is found in patients responding to therapy.

12. The epithelial cell foot process lesion is the first pathologic change recognizable in the idiopathic nephrotic syndrome (nephrosis) and is present when renal glomeruli appear to be normal by light microscopy. Abnormality of structure of the endothelial cells and lamina densa are visible by electron microscopy of renal biopsy specimens obtained later in the course of the nephrotic syndrome and are correlated with the proliferative and membranous changes recognized by light microscopy.

13. Hypotheses concerning the mechanisms of proteinuria and the possible relationship of glomerular structure to the nephrotic syndrome are discussed.

The technical assistance of Miss Joyce Lounberg and Mrs. Ruth Schurr is gratefully acknowledged.

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Studies on the pathogenesis of nephrotic edema

With particular emphasis upon changes in renal hemodynamics and the metabolism of electrolyte and proteins

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CHICAGO, ILL.

THE accumulation of edema in the nephrotic syndrome† begins with excessive renal tubular reabsorption of sodium and water relative to the amounts being filtered by the glomeruli. This reabsorptive pattern is in some way related to the increased glomerular permeability to plasma proteins, particularly albumin.[1, 2] Edema is sustained and augmented principally by extrarenal factors. Attempts by the kidney to maintain the volume and electrolyte concentrations of the body fluids are impaired by underlying renal disease, by medically imposed distortions of load requiring a flexibility of response of which the kidney is not capable, by local and extrarenal cellular factors which condition the activities of renal tubular cells, and by complicated hormonal and hemodynamic events which present a further challenge to the already disorganized kidney. Given the initial reversible renal lesions, the events leading to the development of edema are taken as evidence of inadequate renal function; however, they may also be interpreted as attempts by the kidney to maintain the body fluids against an almost overwhelming array of obstacles. It is the purpose of this paper to present data accumulated during

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The studies reported herein were supported by Grants H-748 C1-4, A-251, AH-807, A-1325 C, A-1325 C1 of the United States Public Health Service, National Institutes of Health, Bethesda, Md. Several of the clinical studies were supported in part by grants from the New England Nephrosis Foundation.

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†*The nephrotic syndrome is defined here as a primary renal disease involving the entire nephron, particularly the glomerular basement membranes, which appear thickened when stained and examined by light microscopy and which show loss of pedicels, coalescence of the podocytes, and possibly breaks in the basement membrane by electron microscopy. The disease has an insidious onset without antecedent nephritis, usually in the 2- to 5-year age group. It is characterized by gross edema, with ascites, a chronic course with remissions and relapses over a period of several years, and a tendency to the development of severe bacterial infections. In relapse the patients exhibit heavy proteinuria, marked hypoproteinemia with striking deficits of plasma albumin and certain globulins, greatly increased plasma beta lipoproteins with hyperlipemia and characteristic abnormalities in the metabolism of plasma and tissue proteins. Nephrotic children may variably manifest transiently decreased, normal, or increased renal functions, microscopic hematuria, cylindruria, intermittent hypertension, and azotemia, particularly during infections or during therapy with ACTH or corticosteroids. Currently, 20 to 30 per cent of nephrotic children die within 5 years of the onset of their disease. Complete recovery may occur in 40 to 50 per cent of affected children during this interval; in others some signs of renal disease may persist for many years. In our opinion, sufficient evidence is available to discard the practice of classifying certain cases in children as lipid nephrosis and others as chronic nephritis in accordance with the clinical or laboratory findings at any particular time. We prefer to consider under the general term, "the nephrotic syndrome," all cases conforming to the above criteria as varying manifestations of a single disease process of unknown etiology.*[3]

the course of a long series of studies on the nephrotic syndrome and to attempt some integration of these in order to define general patterns of excretion of salt and water by the kidneys and to determine the relation between the disturbed metabolism of ions and proteins and the pathogenesis of nephrotic edema.

METHODS AND MATERIALS

Unless otherwise noted for special studies, patients were selected from a large group of children with the nephrotic syndrome being followed and treated at the Children's Hospital Medical Center, Boston, from 1944 to 1956.* Selection usually was based upon the problem to be studied, the clinical impression and laboratory evidence that the child was an "average" nephrotic patient without serious renal insufficiency. Girls were favored for studies requiring catheterization of the bladder and boys for those requiring detailed balance collections. Renal function and load response studies, and electrolyte measurements in serum, urine, feces, and other biologic fluids were carried out by slight modifications of standard analytic techniques.[4] All balance collections were completed in the Metabolic Unit and the chemical composition of intake and outgo were determined by direct analysis. Estimations of the distribution of edema fluid and chemical composition of skin and muscle in rats with an experimental nephrotic syndrome were made according to analytical techniques developed or modified in our laboratories.

RESULTS

A. Metabolism of the plasma proteins. As soon as methods for estimation of different plasma protein fractions were introduced into medicine, it was recognized that there was a very severe disturbance of the plasma proteins in the nephrotic syndrome. Marked hypoproteinemia, with a complete reversal of the normal albumin:globulin ratio, and hyperlipemia were characteristic biochemical findings and had to be related in some way to the cardinal clinical manifestations of edema and proteinuria. The observed reduction in serum colloid osmotic pressure to values about *one third* of normal seemed to provide a satisfactory explanation for the edema, on the basis of Starling's law, until it was found that rapid diuresis with loss of edema could occur with only minimal changes in colloid osmotic pressure. Two general theories have been proposed to explain the disturbances in protein metabolism observed: first, that the disease is mainly metabolic, resulting in altered protein synthesis with excretion of abnormal proteins in the urine; second, that the disease process primarily damages the kidney, with consequent loss of normal proteins in the urine and resultant hypoproteinemia. With the chemical tools available until very recently, the clinical investigator was unable to obtain a satisfactory answer to this problem. What evidence there was seemed to favor the first hypothesis, since the urinary proteins differed in one way or another from the normal plasma proteins when examined by the techniques available.[5, 6]

The development of better methods for the separation of proteins from mixtures, the refinement of biophysical and immunochemical techniques for characterizing them, and the introduction of isotopic labeling to obtain a dynamic picture of protein metabolism have given investigators fairly definitive answers to this central problem in the pathologic physiology of the nephrotic syndrome.

*From 1944 to 1956 several investigators participated in one or more of the studies to be reported. These included Drs. Niilo Hallman, Gretchen Hutchins Moll, Lewis Barness, Weston M. Kelsey, C. Phillips Rance, Nobyuki Nakasone, John James, Elaine Donnellan, Gustavo Gordillo, Silvestre Frenk, Newton Chalk, Jorge Martner, and Francis X. Fellers. The data to be presented largely represent previously unpublished observations which could not have been made except for the stimulation, interest, and diligence of these colleagues, ably assisted by alert, interested house officers and nurses, particularly the metabolism nurses, the Misses Miriam Whyte, Audrey Andrews, Mary Gadway, and Heather Kemp. Finally, the cooperation and faith of the parents and their children should be acknowledged as the essential feature which made these studies possible. The interest and guidance of Drs. James L. Gamble and William Wallace in many of these studies require special acknowledgment. The opportunity to exchange ideas with many investigators at the Annual Conferences on the Nephrotic Syndrome undoubtedly subtly modified the interpretations of some of the data to be published.

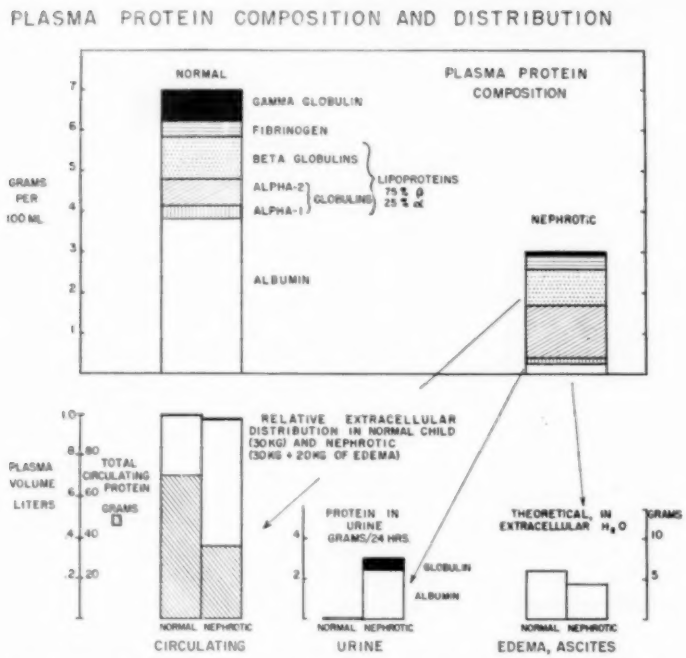

Fig. 1. The nephrotic syndrome is characterized by reduction in plasma albumin concentration, resulting from loss of this protein in the urine and by enhanced catabolism without increased synthesis. Gamma globulin is also lost in the urine. The fractional rate of catabolism of the α-lipoproteins is increased. The hypercholesterolemia and hyperlipemia of the nephrotic syndrome are associated with an increase in the low-density lipoproteins, principally the β lipoproteins. Neither α nor β lipoproteins are lost via the urine in appreciable amounts. The reduction in plasma albumin concentration accounts for the reduction in circulating albumin. The extravascular albumin pool is not increased in edematous nephrotic children as a result of reduced concentration, despite an expanded volume of distribution.

Using the Tiselius electrophoresis apparatus, Luetscher[7] showed that the protein fractions most reduced in the plasma of the nephrotic patient (albumin and gamma globulin) were those present in the greatest relative amounts in the urine[8] (Fig. 1). Studies in our laboratory by Gitlin[9] have shown that the proteins deficient in the plasma are found in decreased amounts and at very low concentrations in the edema and ascitic fluid, so the size of the body pool is presumably greatly decreased.

Soon after Luetscher's important investigation, concentrated human serum albumin was made available through the development of a practical process for the large-scale fractionation of human plasma by Cohn and his associates.[10] Although it proved disappointing as a therapeutic agent in the nephrotic syndrome, human serum albumin has been a valuable tool for investigation of some of the physiologic disturbances in this disease. Infusion of albumin prepared from the pooled plasma of healthy human donors demonstrated that this normal plasma protein was not retained by the kidneys of patients with the nephrotic syndrome. Accompanying its infusion as a hyperoncotic solution, there was a rapid increase in plasma volume, as interstitial fluid was drawn into the circulation, and a sharp rise in the total amount of protein and in the relative

amount of albumin being excreted in the urine (Fig. 2). The inability of the nephrotic kidney to retain normal albumin was further substantiated by the demonstration that the albumin in the plasma, ascitic fluid, edema fluid, or urine of children with the nephrotic syndrome was indistinguishable by quantitative immunochemical analysis from albumin crystallized from fraction V of pooled normal human plasma.[9] These findings strongly support the concept that there is leakage of normal plasma proteins through a damaged kidney rather than the formation of abnormal proteins and their consequent excretion by a relatively normal kidney.

So-called "salt poor" albumin solution,[11] with an effective osmotic pressure five times that of plasma and a sodium concentration in the diluent approximately equal to that of plasma, seemed to offer a very promising diuretic agent for treatment of the troublesome edema of the disease. Careful studies showed that whereas, in many patients, the repeated administration of large doses of albumin would induce a diuresis, this generally produced very little lasting increase in the serum albumin concentration, and edema tended to reaccumulate as soon as albumin infusions were discontinued. In a large-scale trial of albumin distributed by the Red Cross, approximately half of the patients with the nephrotic syndrome had a diuretic response, but in a small group of adults with edematous glomerulonephritis, Thorn and his associates[12] were generally able to induce a satisfactory diuresis by continuous infusion of 50 Gm. of albumin per day as a 10 per cent solution in 6 per cent glucose.

Detailed physiologic studies of the effects of albumin infusion in nephrotic patients by Lauson and his colleagues[2] demonstrated an increase in plasma volume and an increased glomerular filtration rate to which they attributed the diuresis usually observed. Our own early observations in a nephrotic child showed that intravenous infusion of albumin first resulted in a water diuresis followed shortly by an increased output of water and electrolytes (Fig. 3). This observation was elaborated by Luetscher[13] in a careful study of the effects of albumin infusions. All of his patients had a prompt water diuresis soon after the infusion but a significant diuresis and loss of edema occurred only when a marked increase in the excretion of sodium and chloride followed this initial response. Luetscher carefully followed the fate of the infused albumin and, although most of it was lost in the urine within a few days, a small and varying portion was retained in the body protein pool.

The failure of certain patients to excrete sodium in response to albumin infusion led Luetscher to examine the urine of his patients for salt-retaining substances. He was able to demonstrate the presence of steroid-like compounds in the urine of edematous patients which, when suitably prepared and injected,[14] would induce sodium retention in rats. Such sodium-retaining substances were found in the urine of edematous nephrotic patients in increased amounts and their output diminished sharply at the time of diuresis, whether this was induced by albumin or cortisone.[15] Subsequent studies[16, 17] have identified this sodium-retaining factor as aldosterone. Thus it seems probable that albumin infusion, through its effect on plasma volume and glomerular filtration rate, suppresses antidiuretic hormone secretion, thus initiating a water diuresis, and may diminish the secretion of aldosterone and thus limit the reabsorption of sodium by the kidney in certain patients.

During hospitalization of children with the nephrotic syndrome, accidental cross-infection frequently occurs. The observation that an intercurrent measles infection was followed by a striking remission led us to try intranasal inoculation of throat washings taken from patients with measles in the Koplik spot stage in a group of nephrotic children with protracted and severe edema.[18] A remission occurred in 9 of 12 children who developed measles following inoculation. Comparable results were obtained in a similar trial in Cincinnati.[19] In contrast to the diuresis produced by albumin infusion, diuresis after measles infection differed in

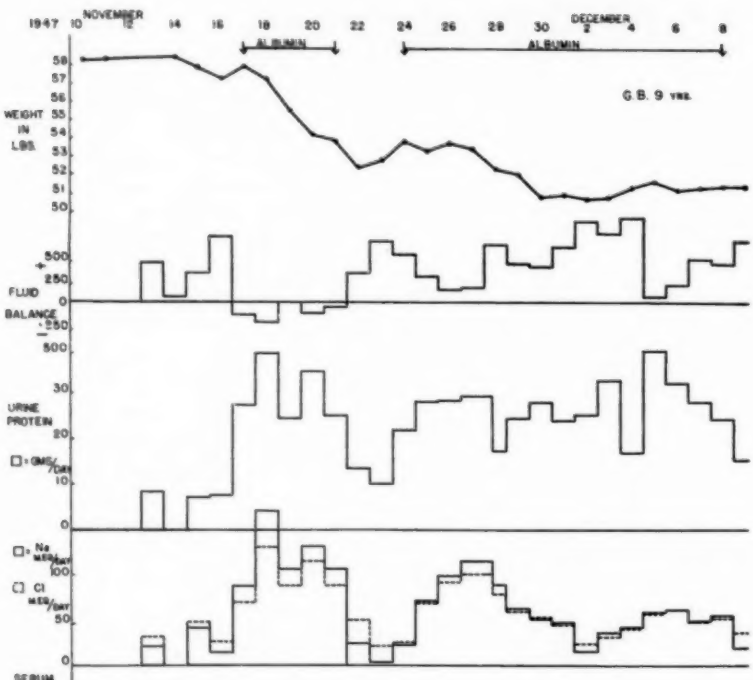

Fig. 2. The effect of daily intravenous infusions of 25 Gm. of albumin as a 25 per cent solution upon body weight, water balance, proteinuria, urinary sodium and chloride excretion, and the serum protein level of a 9-year-old boy with the nephrotic syndrome in whom albumin was an effective diuretic agent. Edema was no longer apparent after November 30. Note particularly: (1) the reaccumulation of edema as shown by a rise in weight, positive water balance, fall in urinary sodium and chloride excretion, as soon as the infusions were discontinued between November 21 and 24; (2) the failure of the infusion of a total of 500 Gm. of albumin in 22 days to raise the serum protein level significantly; (3) the marked increase in urinary protein during the periods when albumin was being administered.

the following ways: (1) Diuresis did not occur immediately but during the period of recovery from measles. (2) Diuresis, when it did occur, was usually more dramatic and rapid (Fig. 4). (3) It was associated with a decrease rather than an increase in proteinuria. (4) It was followed by a period of variable duration (weeks or months) of remission of the major features of the disease (Fig. 5). The rise in serum colloid osmotic pressure, with restoration of the plasma proteins toward normal, which occurred in these remissions, began either simultaneously with or shortly after onset of the diuresis and hence was not responsible for the diuresis (Fig. 6). This led us to postulate some metabolic changes as a cause of the diuresis.[18] Since ACTH or cortisone became available and were used in treatment, the similarity of the results with respect to the frequency with which a diuresis occurs and to the sequence of physiologic events suggests that the metabolic change induced by measles infection may well have been the secretion of increased amounts of hydrocortisone by the adrenal under the stress of infection. There

has been no opportunity to test this hypothesis by direct observation of corticosteroid metabolism in patients with measles. Other severe infections caused by viruses or bacteria have also been followed by diuresis, but less frequently in the case of bacterial infections, perhaps because the severity of these infections has been so greatly diminished by antimicrobial chemotherapy in recent years.

The possibility of isotopic labeling of plasma proteins provided the first opportunity to obtain a dynamic picture of the metabolism of the plasma proteins in the nephrotic syndrome. Methionine labeled with S^{35} was fed to several children with the nephrotic syndrome by Kelley and associates,[20] and its rate of incorporation into their plasma proteins compared with the rate of incorporation in normal children. The results were taken to indicate an increased rate of synthesis, but, in the absence of data on pool size and with the probability of reincorporation of labeled methionine derived from the catabolism of labeled protein, it is very difficult to draw firm conclusions from the data.

Because of these difficulties, our studies have been made with I^{131}-labeled specific plasma proteins. Provided great care is used in the labeling process, significant denaturation of the protein does not seem to occur, and the physiologic behavior of different preparations is consistent. Tracer doses of

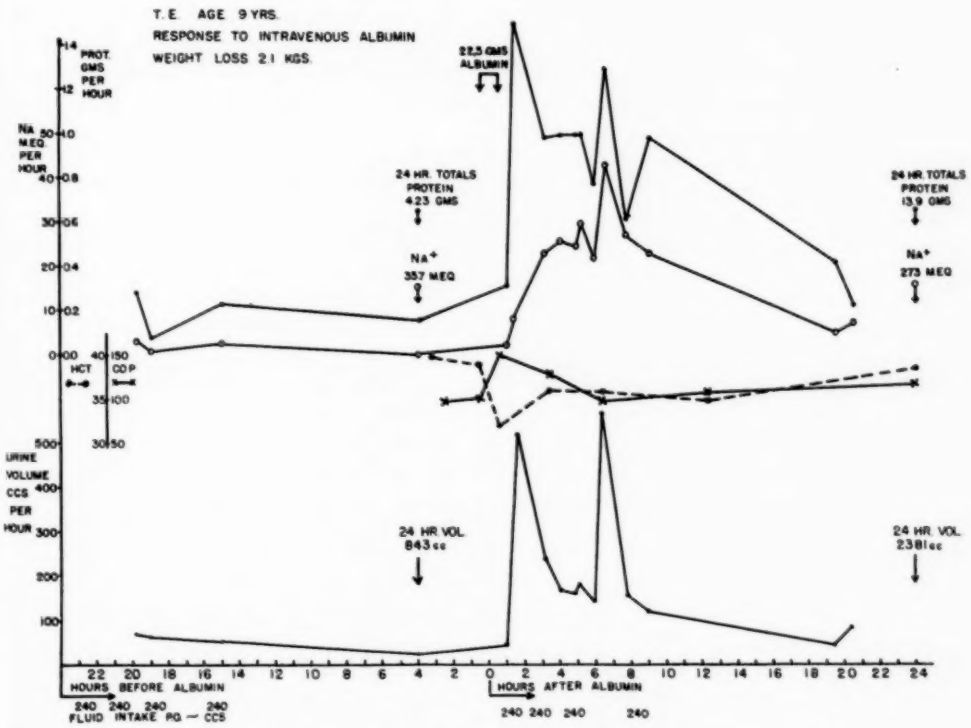

Fig. 3. The sequence of events in a 9-year-old boy with the nephrotic syndrome who had a diuretic response to the intravenous infusion of 22.5 Gm. of albumin as a 25 per cent solution containing approximately 20 mEq. of sodium. The observations cover a 24 hour period before the infusion and a similar period thereafter. Note that the first change was a rise in colloid osmotic pressure and an increase in plasma volume, as shown by a fall in hematocrit from 40 to 32 per cent, which was then immediately followed by a rapid rise in the excretion of water and protein, with a slower rise in the excretion of sodium.

Table I. Turnover half-times of plasma proteins in children with nephrotic syndrome as estimated from disappearance of intravenously injected tracer doses of I^{131}-labeled proteins

Protein	*Half-time in*		*Relative urinary loss*
	Normal or nephrotic in remission (days)	*Active nephrotic syndrome (days)*	
Albumin*	12	1.8-2.3	++++
α-lipoproteins†	4.4-4.8	2.3-3.7	+
β_1-metal combining globulin*	—	1.7-2.5	+++
S_f 3-9 β-lipoproteins†	3.0-3.4	2.5-2.8	±
γ-globulins	2.2	3.3-4.1	+++

*Data from Gitlin, Janeway, and Farr.[21]

†Figures refer to peptide moiety of lipoproteins, which was the portion labeled with I^{125}. Data from Gitlin and co-workers.[22]

I^{131}-labeled proteins have two great advantages for the purposes of such studies: (1) as the labeled protein is catabolized, I^{131} is released and promptly excreted in the urine as radioiodine, provided the thyroid has been blocked by suitable doses of iodine prior to injection of the labeled protein, thus providing a measure of catabolism of the proteins; (2) the label is not reincorporated into proteins being synthesized by the patient under investigation. Consequently, any radioactivity present in the body fluids after injection of I^{131}-labeled protein represents the labeled protein itself.

For dynamic studies of plasma protein metabolism 6 children in various stages of the nephrotic syndrome in the Medical Department of the Brookhaven Laboratories were selected. One was in complete remission, except for a trace of proteinuria, 2 were in partial remission with characteristic laboratory findings of nephrosis but only minimal edema, and 3 were in full relapse with generalized edema. All remained in the same condition throughout the period of study lasting 2 months, that is, they were in the "steady state." The metabolism of albumin, of gamma globulin, and, in 3 patients, of β_1-metal combining globulin (transferrin) were studied, with the use of tracer doses of I^{131}-labeled proteins. Serial measurements of radioactivity in the blood and of radioprotein and radioiodide in the urine yielded curves of disappearance of labeled protein from the blood and a measure of the amount of labeled protein lost through proteinuria and through catabolism. Table I gives the data as obtained by calculation from these studies and shows the greatly shortened half-time of disappearance for all three proteins in the patients with active disease as compared with the patient in remission, in whom values were normal. In relapse, the shortened half-life of the labeled proteins could be attributed both to increased catabolism and to loss as protein in the urine. Thus far, no satisfactory explanation for this increased catabolism has been found.[21]

In a second series of studies, the peptide (not the lipid) moieties of β-lipoproteins of differing S_f classes (higher density and lower density) were labeled with I^{131} and similar studies carried out in another group of patients. No exchange of the label was observed between the alpha and the beta lipoproteins, although their lipids may exchange very readily. The beta lipoproteins are markedly increased in amount in the nephrotic syndrome, thus accounting for the hyperlipemia and hypercholesterolemia.[22] Although interpretation of the data upon the metabolism of the peptide components of the beta lipoproteins presents many difficulties, there can be no question that these proteins, which are present in increased amounts in the blood, are not excreted in the urine and have, if anything, a lengthened half-time (Table I). Thus in the case of the majority of plasma proteins with their shortened half-life due to urinary loss and increased catabolism and of the beta lipoproteins with their lengthened half-lives and retention in the blood, these dynamic studies provide adequate descriptions of the metabolic alterations which account for the dysproteinemia which is so characteristic of the nephrotic syndrome.

These studies provide convincing evidence of the magnitude of the urinary losses and

increase in catabolism of the labeled proteins injected. In using I^{131}-labeled proteins we assume, first, that the metabolic behavior of the labeled protein reflects the metabolism of the patient's endogenously produced protein and, second, that the splitting off of I^{131} from the tyrosine of the labeled protein is an accurate reflection of catabolism of the protein molecule and particularly of the patient's own protein. If these assumptions are valid, and we believe that they are reasonably so, then we may infer the metabolism of the patient's own proteins from the measured behavior of these tracer doses. Since the patients were in the "steady state" throughout these studies, it seems fair to assume that the rates of synthesis, of catabolism, and of urinary loss, and hence the size of the body pool of each of the proteins studied, remained relatively constant throughout the study period. Under these circumstances, the rate of synthesis necessary to maintain the body pool at constant size would equal the sum of the rates of urinary loss and catabolism, and hence the rates of synthesis for each of the proteins studied can be calculated and compared with those known from similar studies upon normal subjects in the literature.

In patients with an active nephrotic syndrome, rates of synthesis for albumin and β_1-metal combining globulin appear to be normal, for gamma globulin slightly increased over normal, and for beta lipoproteins markedly increased.

Studies such as these give us a dynamic description of the metabolism of the different plasma proteins, but do not give us an *ex-*

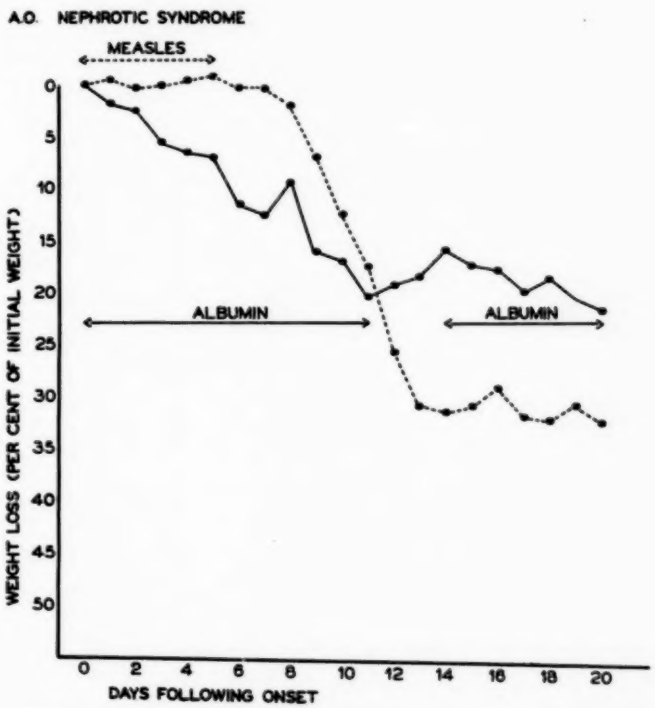

Fig. 4. Comparison of diuretic response (as shown by weight loss) following measles (dotted line) with that accompanying the daily infusion of albumin (solid line). Measles is followed by a dramatic diuresis beginning a few days after the disease, while albumin infusions produce only transient diuresis with reaccumulation of edema if the infusions are stopped.

Table II. Changing renal functions with spontaneous, dextran, or ACTH-induced diureses (all data per square meter)

Date	*Date of onset of disease and age at onset*	*Duration of disease (months)*	*GFR (ml./ min.)*	*RPF (ml./ min.)*	*Tm (mg./ min.)*	$\frac{GFR}{RPF}$	$\frac{RPF}{Tm}$	$\frac{GFR}{Tm}$
M. P. ♀ No. 356118								
1/22/51*	9/49	14	42	271	34	0.16	7.9	1.2
2/12/51†		15	48	204	24	0.24	8.4	2.0
3/13/52‡	(3 1/12)	28	55	207	31	0.27	6.7	1.8
4/30/53		41	65	240	31	0.27	7.8	2.1
6/17/54		55	62	214		0.29		
5/3/56		78	54	187	37	0.29	5.1	1.5
A. S. ♀ No. 370905								
3/19/51*	2/51	1	59	345	60	0.17	5.8	1.1
4/5/51†		2	80	302	89	0.26	3.4	0.9
6/12/51‡	(6 1/12)	14	97	638	75	0.15	8.5	1.3
7/16/53		27	129	835	63	0.15	13.3	2.0
8/30/54		40	59	393	56	0.17	6.9	1.1
5/17/56§		63	76	306	57	0.24	5.3	1.3
C. P. ♀ No. 355028								
1/30/50‖	1/50	½	12	125	14	0.09	9.2	1.1
6/5/50*		5	19	87	8	0.22	9.5	2.3
6/22/50†	(1 3/12)	5	46	215	22	0.21	9.7	1.9
6/11/51‡		17	60	223	40	0.27	5.6	1.5
6/19/52		29	71	303	45	0.22	7.3	1.6
5/28/53		40	104	322	58	0.33	5.5	1.8
6/14/54		53	71	368	60	0.22	8.0	0.9
6/14/56§		77	60	218	32	0.28	6.9	1.9
S. J. ♀ No. 354053								
3/23/50*	1/50	2	35	296	30	0.12	9.9	1.1
4/30/50†		3	52	264	30	0.20	8.9	1.6
6/8/50	(4 5/12)	5	54	174	37	0.31	4.7	1.3
11/27/50*		10	27	269	30	0.10	9.0	0.9
12/18/50†		11	38	224	44	0.17	5.1	1.1
7/2/51¶		18	16	118	9	0.13	12.8	1.4
died 12/52								
D. F. ♀ No. 364598								
10/16/50*	7/50	3	62	216	50	0.31	4.3	1.2
11/1/50†		4	57	170	43	0.35	3.9	1.3
9/22/52‡	(3 1/12)	26	79	503	43	0.16	11.8	1.8
6/12/53		35	59	299	34	0.20	8.8	1.7
6/22/54		47	18	143	26	0.12	5.5	0.5
10/11/54#		51	20	168	38	0.12	4.4	0.5
3/29/56		68	16	82	15	0.19	5.6	1.0
H. S. ♂ No. 346666								
2/6/50	4/49	10	24	115	7	0.21	15.8	2.9
6/29/50*		14	31	129	6	0.23	22.5	5.0
6/29/51‡	(3 9/12)	26	46	163	33	0.29	4.9	1.0
7/2/52		38	43	192	35	0.23	5.5	1.2
7/2/53		50	75	283	—	0.26	—	—
8/17/54§		63	57	289	30	0.20	9.5	1.7
J. M. ♂ No. 359828								
5/25/50*	2/50	3	19	214	28	0.08	7.5	0.7
6/15/50†		4	79	279	37	0.24	7.5	1.5
6/8/53‡§	(2 9/12)	40	69	444	52	0.18	8.5	1.2

*Before ACTH.

†After ACTH diuresis.

‡Remission of 1 year.

§In remission, or apparently well, 1956.

‖Onset 2 weeks earlier.

¶Uremic with NPN > 200 mg. per cent and died 12/52.

Table II. Cont'd

Date	*Date of onset of disease and age at onset*	*Duration of disease (months)*	*GFR (ml./ min.)*	*RPF (ml./ min.)*	*Tm (mg./ min.)*	$\frac{GFR}{RPF}$	$\frac{RPF}{Tm}$	$\frac{GFR}{Tm}$
C. B. ♀ No. 397660								
11/19/53	9/52	11	18	163	—	0.11	—	—
3/13/54		15	25	290	—	0.09	—	—
6/24/54	(2 1/12)	18	15	100	16	0.16	6.1	0.96
Died 11/54								
S. L. B. ♀ No. 387366								
12/30/52	2/52	8	57	331	—	0.17	—	—
4/18/53† dextran		14		147	—			
12/28/54	(10 9/12)	32	73	259	34	0.28	7.7	2.2
4/26/56§		38	106	315	72	0.22	4.4	0.97
B. L. ♀ No. 404685								
10/14/53*	6/53	4	27	350	89	0.08	3.9	0.3
10/22/53*		4	27	167	23	0.16	7.3	1.2
11/9/53†	(2 9/12)	5	64	259	—	0.25	—	—
9/2/54		15	66	290	42	0.25	7.0	1.5
4/5/56§		22	64	211	52	0.31	4.5	1.2
L. W. ♀ No. 390478								
10/6/52*	9/52	1	18	312	38	0.06	8.3	0.5
10/30/52†	(6 1/2)	2	78	412	54	0.19	7.6	1.4
8/19/54		22	74	319	49	0.23	6.5	1.5
5/24/56§		42	55	330	57	0.17	5.8	1.0
S. W. ♀ No. 366212								
2/12/53	8/50	30	20	213	32	0.09	6.7	0.6
6/7/54		46	16	173	16	0.09	10.8	1.0
4/19/56	(4 10/12)	68	19	89	19	0.21	4.6	1.0
C. F. ♂ No. 409951	6/53							
1/12/54*		6	46	354	20	0.31	17.7	1.8
1/19/54 (after dextran diuresis)		6	85	406	31	0.21	13.1	1.2
2/4/54†		7	72	241	56	0.30	4.3	1.3
9/14/54**		14	50	404	48	0.12	8.5	1.0
5/31/56	(3 7/12)	35	76	379	57	0.20	6.6	1.3
E. M. ♀ No. 390585								
9/4/52	1/49	46	30	254	46	0.11	5.5	0.7
4/27/53‡		53	59	432	51	0.14	8.5	1.2
8/12/54§	(2 6/12)	69	65	335	41	0.19	8.2	1.5
L. C. ♀ No. 343049								
1/18/50	10/49	3	56	285	45	0.19	6.3	1.3
6/14/53††	(3 8/12)	44	51	384	47	0.13	8.2	1.1
L. A. ♀ No. 359815								
5/22/50	4/50	1	53	208	43	0.23	4.9	1.0
6/21/51††	(2 2/12)	14	64	252	40	0.26	6.3	2.1
M. N. ♀ No. 395582								
1/29/53	12/52	1	54	298	43	0.18	6.9	1.0
5/1/53 dextran	(2 5/12)	6	49	185		0.26		
5/29/53 dextran		7	68	308		0.22		
9/23/54†		20	80	284	37	0.28	7.7	2.2
6/21/56§		41	52	242	38	0.21	6.4	1.4

‡Relapse.

**Daily ACTH from 2/7 to 5/27/54, and every other day thereafter to 3/8/55, "intermittent" cortisone thereafter.

††Spontaneous diuresis.

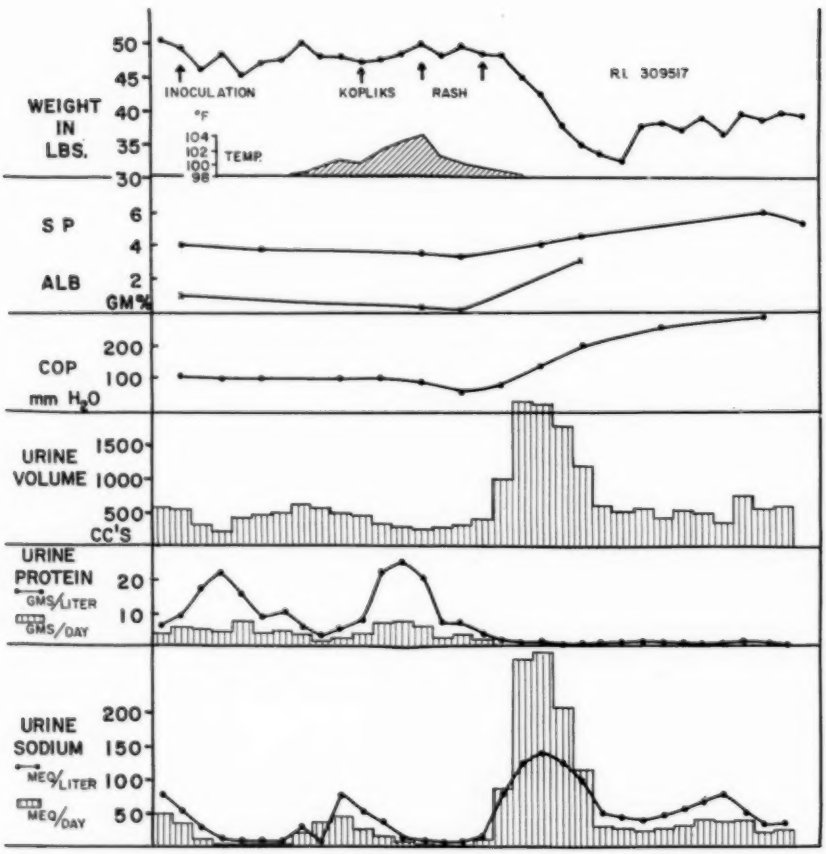

Fig. 5. The effect of an attack of induced measles upon a boy with the nephrotic syndrome. Inoculation was by nasal instillation of throat washings from a case of measles in the Koplik spot stage taken in broth containing penicillin and streptomycin. Chart shows, from above downward, changes in body weight, serum protein, serum albumin, colloid osmotic pressure of serum, urinary volume, urinary protein excretion, and urinary sodium excretion. All edema disappeared during a 4 day period of diuresis, and in this case the remission was complete, although not permanent.

planation for the metabolic changes. The loss of various proteins in the urine seems to be explicable on the basis of changes in the glomerular basement membrane, which according to some observers show discontinuities or "holes" in the thickened membrane in electron microscopic sections. The fact that the plasma proteins excreted in the greatest amounts are those with the lowest molecular weights, while those which are not lost in appreciable amounts (fibrinogen and β-lipoprotein) have the highest molecular weights lends credence to this interpretation. Other observers are unable to demonstrate "holes" in the thickened basement membrane and believe some form of pinocytosis accounts for the leakage of proteins through the glomerulus.[23] Final interpretation of the glomerular changes seen with the electron microscope will require better techniques and further study. No satisfactory explanation for the increased body catabolism of proteins in addition and identical to those which are lost in the urine,

or for the increased synthesis of beta lipoproteins has yet been advanced. Animal experiments suggest that under normal conditions it is the cells of the reticuloendothelial system which play the major role in catabolism of the plasma proteins.[24]

Although the dynamic studies upon children with the nephrotic syndrome seemed to give rather consistent results, a similar study of a group of adults with the nephrotic syndrome[25] did not demonstrate this increased albumin catabolism. Hypoproteinemia in these patients, who were much less edematous than the children, was primarily due to urinary loss and in no patient to a decrease in albumin synthesis as well. Whether these differences are due to differences in response to the same process at different ages, to the relative mildness of the disease at the time these adults were studied, or to the fact that the disease process in adults is a different one is not known.

Thus, we can summarize by saying that, although modern physiologic and biochemical investigation has given us a far more accurate description of the metabolic mechanisms which account for the dysproteinemia of the nephrotic syndrome than we previously possessed, there is much that still cannot be explained. However, the biochemical and physiologic studies point, as do the pathologic studies, to the change in the glomerular basement membrane as the primary lesion, which permits the escape into the urine of proteins up to a certain molecular size. These urinary losses of practically all the plasma proteins except fibrinogen and beta lipoproteins, but particularly of albumin, potentiated by the unexplained hypercatabolism of these proteins and uncompensated by a marked increase in their rate of synthesis, result in a lowering of plasma colloid osmotic pressure. The subsequent circulatory and hormonal (ADH and aldos-

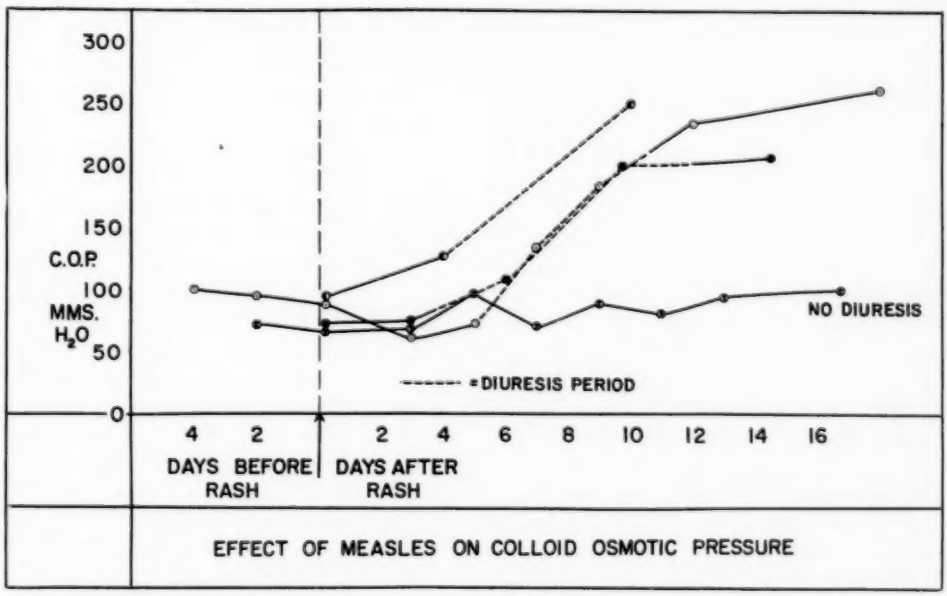

Fig. 6. Relationship between the rise in serum colloid osmotic pressure following measles and the timing of the diuresis in 3 patients who had a remission following measles. Note failure of colloid osmotic pressure to rise in one patient in whom no diuresis or decrease in proteinuria occurred. There may be a very slight rise in colloid osmotic pressure prior to diuresis, but the real rise accompanies the diuresis, suggesting that both phenomena are dependent upon some more basic change rather than dependent upon one another.

terone) adjustments and the concomitant changes in renal functions and metabolism of electrolytes and water which give rise to the edema will form the principal subject of the succeeding sections.

B. Renal functions and metabolism of electrolytes.

1. Renal functions. Patterns of renal functional response vary markedly in children with the nephrotic syndrome. It has been suggested that particular levels of glomerular filtration, TmPAH, or filtration fraction may have prognostic implications.[26] Serial measurements of discrete renal functions before and after diuresis (ACTH induced, after dextran, or spontaneous) are given in Table II. Analysis of the data in Table II emphasizes the extreme variability and lack of clear-cut prognostic implication from any given parameter of renal function measured early in the course of the nephrotic syndrome in children. This is consistent with the results of previous studies[3, 27, 28] and avoids the tautology of assigning a diagnosis of "nephrosis" only to the patients who do not have hematuria and have normal clearances or assuming that patients who die from renal disease must have had glomerular nephritis, not "nephrosis." Extreme reduction of glomerular filtration out of proportion to that of renal plasma flow* very early in the disease may be altered with diuresis, and gradual return of normal patterns of renal function may occur, as in Patient C. P. On the other hand, early "supernormal" glomerular filtration and renal plasma flow do not necessarily indicate a favorable prognosis (Patient D. F.). Nor are the ratios of the several parameters of renal function of great prognostic import early in the course of the disease. For example, S. J. ultimately succumbed from progressive renal failure although values for GFR and $\frac{GFR}{RPF}$ were within normal limits on several occasions and those for $\frac{RPF}{Tm}$ were generally well preserved, as were those for $\frac{GFR}{Tm}$. By contrast, A. S. recovered, although values for $\frac{GFR}{RPF}$ and $\frac{RPF}{Tm}$ generally were reduced. In general, frequently observed transient reductions in several moieties of renal function, including the filtration fraction, within the first 18 months of the disease appear to have less prognostic significance than similar reductions occurring later in the course of the disease.

As suggested by physiologic evidence[3, 31] and substantiated by recent morphologic studies,[32, 33] the renal manifestations of the nephrotic syndrome reflect a varying balance between reversible changes in glomerular basement membranes and tubular cells and gradually superimposed, irreversible obliteration of glomeruli, and subsequently of entire nephrons, following gradual occlusion of glomerular capillaries with progressive thickening and impermeability of their membranes.

The role of interstitial edema in altering renal function is not well defined. Ferris[34] points out that interstitial pressure is not the same everywhere throughout the kidney, since the kidney as a whole does not behave like a completely fluid system. The interstitial renal pressure has been estimated at 10 to 18 mm. of mercury and appears to be proportional to the renal venous pressure.[35] In the presence of renal edema the interstitial pressure may be substantially greater than the venous pressure. It is obvious that expansion of interstitial volume within a tissue confined by a tight capsule may cause extrinsic compression of delicate capillary walls and thereby increase resistance to flow. The recent countercurrent hypothesis of urine concentration[36, 37] suggests that interstitial edema might serve to decrease the rate of flow through vasi rectae and tubular lumina in the region of the loop of Henle.

*The validity of estimates of renal plasma flow in the presence of renal disease is primarily dependent upon the effective extraction of the test substance as indicated by Cargill[29] and Bradley.[30] It appears that low PAH clearances may be associated with exponentially decreasing values for PAH extraction, hence apparent renal plasma flow as measured may be considerably lower than true renal plasma flow unless corrected for reduced extraction of the test substance.

Table III. Immediate effect of paracentesis on renal functions

Patient	*Relation to paracentesis*	*SA (M.²)*	*Weight (Kg.)*	*Intra-abdominal pressure (mm. H_2O)*	*No. of periods*	*GFR (ml./min./M.²)*	*RPF (ml./min./M.²)*	*F.F.*
J. K.	Before	0.54	18.3	250	4	11	36	0.31
♂	After		15.1	70	5	9	25	0.36
M. P.	Before	0.68	24.53	110	3	52	262	0.20
♀	During				3	63	289	0.22
	After		24.12	100	3	47	241	0.20
R. K.	Before	1.10	(5.67 L. ascitic fluid removed)	185	5	12	84	0.15
♂	After				5	13	103	0.13
M. M.	Before	0.83	27.57		3	26	106	0.24
♂	After				5	29	101	0.29
A. C.*	Before	0.8	21.0	240	3	70	159	0.44
♀	After		(5.67 L. ascitic fluid removed)	25	4	61	159	0.38

*Ascites due to congenital atresia of bile ducts, not nephrotic syndrome.

Table IV. Effect of diuresis on plasma volume and total circulating plasma protein (7 children)

	Plasma volume (L./M.²)	*Plasma proteins*		*GFR (inulin) (ml./min./M.²)*
		Concentration (Gm./100 ml.)	*Total circulating (Gm./M.²)*	
Before	1.17 ± 0.15*	3.5 ± 0.6	40.7 ± 12.8	35 ± 17
After	1.28 ± 0.24	4.4 ± 0.4	56.3 ± 9.4	56 ± 16

*Mean for group ± standard deviation, σ.

This, in association with the expanded volume of the potential interstitial phase, may lead to a larger gradient favoring the transport of sodium and water from the tubular fluid. While no data are available, it is conceivable that this might be a major factor in the oliguria so characteristic of this syndrome.[38]

The availability of adrenocorticotropic hormone (ACTH) and corticosteroids has made possible not only systematic treatment of the nephrotic syndrome but also the induction of a predictable diuresis in most patients, a feature of therapy which has permitted observations of changes in renal function associated with diuresis. Glomerular filtration rate and filtration fraction tend to improve with diuresis.[1, 3, 15, 26, 28, 39-41] Improvement in glomerular filtration rates and renal plasma flow is not only characteristic of steroid-induced diuresis but seems to occur with diuresis from any cause, including spontaneous diuresis, albumin-induced diuresis,[42, 43] dextran-induced diuresis,[44] etc. (Table II). The nature of the renal hemodynamic change with diuresis is difficult to assess since calculations of renal hemodynamic parameters[45] are based upon the oversimplified concept of flow in simple tubes modified by variations in hydrostatic pressure, colloid osmotic pressure, viscosity, and loss of fluid and protein from renal capillaries.

As blood traverses the glomerular capillaries the colloid osmotic pressure will rise in consequence of the increasing concentration of protein due to the ultrafiltration of water at the glomerulus. Similarly, the colloid osmotic pressure will decrease in the peritubular capillaries at the sites of fluid

reabsorption. Filtration equilibrium probably is not reached in either the glomerular or peritubular capillaries. Gomez assumes that the locus of the shifting hydrostatic point of equality between the opposing hydrostatic and colloid osmotic pressures should occur in the proximal end of the efferent arteriole with continued fall in pressure distally to promote maximal filtration in the glomeruli and maximal reabsorption in the peritubular capillaries. Filtration pressure averages about 60 per cent of the mean arterial blood pressure. Filtration, and renal circulation, may be divided into various functional segments, each arranged in series and each contributing its quota of resistance between renal artery and vein. In general, variations in the resistance offered by any one segment are presumed to be caused by variations in the total cross-sectional area of that segment. Fig. 7

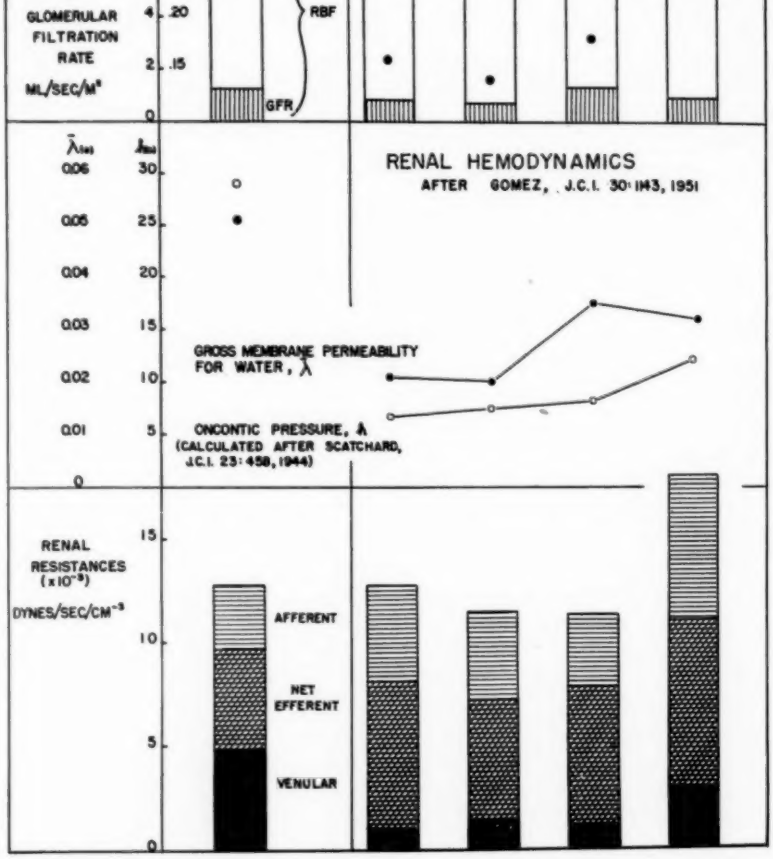

Fig. 7. For legend see opposite page.

illustrates renal hemodynamics characterizing diuresis in 4 nephrotic children as compared with 3 normal children. If the net implication of the various balances in the renal circulation is to maintain glomerular-tubular balance which, in turn, is designed to effect appropriate regulation of salt and water balance, the observations of Fig. 7 clearly indicate that diuresis is associated with a striking readjustment in all vascular segments.

While such calculations cannot be interpreted literally, they provide some insight into the changes occurring in a given patient from time to time. In the top portion of the figure, glomerular filtration rate and renal blood flow are expressed in the somewhat unfamiliar term, milliliters per second per square meter. Diuresis is associated with an increase in both renal plasma flow and glomerular filtration rate and a slight increment in filtration fraction. In subsequent measurements, made one to 2 weeks following diuresis, it is apparent that the elevation of glomerular filtration is partially sustained but that a reduction in renal plasma flow occurs with consequent elevation of the filtration fraction.

Fig. 7. Renal hemodynamic changes associated with diuresis in the nephrotic syndrome were calculated from observed values for renal function in 4 nephrotic children and compared with those in 3 normal, nonnephrotic children of similar age. Studies on the edematous nephrotic patients, 2 7/12 to 6 3/12 years of age, were completed immediately before ACTH therapy began, on the fourth to the fifth day of therapy, on the ninth to the eleventh day, at the onset of diuresis, and, finally, about one week later when diuresis was completed and the patients were no longer edematous. ACTH therapy was given for 10 days. The calculated data from which the middle and bottom sections of the chart were constructed were obtained according to Gomez[45] and Scatchard[47] as follows:

$\overline{\lambda}$ = coefficient gross permeability for water

$$= \frac{g}{(Pg\text{-}h\text{-}H)}$$

q = GFR in ml. per second

$P_m = P_{diast.} + K\ (P_{syst.} - P_{diast.})$ where $K = 0.44$

P_g = mean glomerular pressure = ⅔ mean aortic pressure

h = mean colloid osmotic pressure

$$= C_{Gm./ml.}\ \frac{\frac{310}{298} \times \dot{2}68\ [\ 1\text{-}0.42\ (\dot{0}.85)\ ]}{1 - (0.4 + 0.9\ pH)C}$$ where C = protein concentration

$$H = \text{intracapsular (interstitial) pressure} = H = \frac{Rv\ Pm + (Ra + R'E)\ Pv - \tilde{n}o}{}$$

where $\tilde{n}o$ = minimum colloid osmotic pressure

Q = afferent renal blood flow (= renal blood flow)

P_T = mean peritubular capillary pressure

P_v = renal venous pressure (assumed 10 mm. Hg)

R = total renal resistance (dynes/sec./cm.$^{-5}$)

R_A = afferent resistance

R_E' = net efferent resistance

R_V = venular resistance

$R = RA + R'E + RV$

$$R = \frac{P_m - P_v}{Q} \times \frac{(\text{Wt. } 0.1 \text{ cc Hg} \times G)}{(\quad \text{flow} \quad)} \times \frac{1.354 \times 980.6}{\text{cm.}^3\text{/sec.}} = 1{,}328 \text{ dynes/sec./cm.}^{-3}$$

$$R_A = \frac{P_m - P_g}{Q} \times 1328$$

$$R_E = \frac{P_g - P_t}{Q - q} \times 1328$$

$$R_V = \frac{P_t - P_v}{Q} \times 1328$$

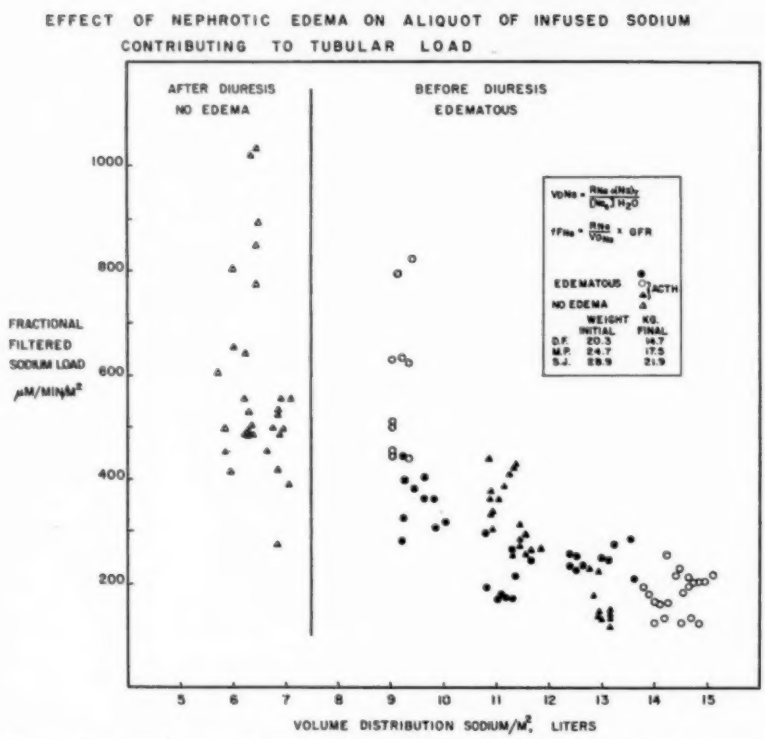

Fig. 8. The quantity of infused sodium which achieves filtration in a short interval (< 3 to 4 hours) during or after intravenous injection (fractional filtered sodium load, fF Na) is inversely related to the volume of distribution of the infusate, at any given level of glomerular filtration. With marked edema, the enlarged volume of distribution reduces the fractional filtered sodium load, as illustrated in 3 edematous nephrotic children. After diuresis, the volume of distribution of the same infused sodium load is decreased, and the fF Na is increased.

The middle portion of the chart illustrates the relation of diuresis to colloid osmotic pressure and of the glomerular membrane to the permeability coefficient for water.

Colloid osmotic pressure was not measured, but calculated according to the technique of Scatchard[46, 47] with suitable substitution of measured albumin concentrations in nephrotic children.[9*] The calculated rise in colloid osmotic pressure following diuresis is rather small and consistent with direct observations (Fig. 6).[18, 48-50] It is clear that elevation of colloid osmotic pressure could hardly account for either diuresis or the improved filtration fraction. The gross membrane permeability coefficient for water (λ) appears to increase about twofold according to calculation, and this improved permeability seems to be sustained following diuresis.

In the bottom section of the chart, the reduced glomerular filtration rate during edema presumably reflects an increase in total renal resistance and a decrease in effective filtering surface, with reduced membrane permeability coefficient for water. The increase in afferent and efferent arteriolar resistances might be consistent with increased glomerular capillary and intracapsular pressures due to thickening of the basement membrane and passage of protein into the subcapsular space. The very low renal

[*]The calculations depend on the fact that albumin, because of its relatively small molecular size, largely accounts for the colloid osmotic pressure of the plasma. This is nicely demonstrated by Squire, Blainey, and Hardwicke.[49]

venular resistance in the peritubular capillaries may result from failure of reabsorption of protein and reduced colloid osmotic pressure as well as from the increased renal interstitial pressures. Treatment resulting in diuresis appears to be associated with a fall in all resistances except renal venular resistance which tends to improve, possibly as a result of reabsorption of interstitial and subcapsular fluid. After diuresis there is a marked increase in all resistances. The renal venular resistance, although increased, is still below normal, possibly because of the improved but still reduced oncotic pressure. Increased afferent resistance could be consistent with residual thickening of the glomerular basement membrane reflected in a decreased renal plasma flow, while the increased efferent resistance suggests active efferent arteriolar constriction with associated increase in hydrostatic glomerular capillary pressure enhancing filtration and accounting for the observed elevation of the filtration fraction.

In addition to the intrarenal resistances influencing glomerulotubular balance, it has been suggested that the accumulation of ascitic fluid may increase abdominal pressure upon the ureters and renal vessels to favor salt retention, presumbly by reducing glomerular filtration[51, 52] or by increasing renal venous pressure.[53, 54] While spontaneous diuresis has occasionally been observed to follow the removal of ascitic fluid in nephrotic children, this is not a common event. That paracentesis has little acute effect on renal functions is indicated in Table III. In these studies abdominal paracenteses were performed during the course of measurements of renal function in edematous, cooperative, calm children. Little change was observed in successive 15 minute periods immediately following paracentesis when each was compared with that preceding it. In one

RELATION OF GLOMERULAR FILTRATION RATE AND SOLUTE EXCRETION TO URINE FLOW DURING OSMOTIC AND ACTH-INDUCED DIURESIS OF NEPHROTIC EDEMA

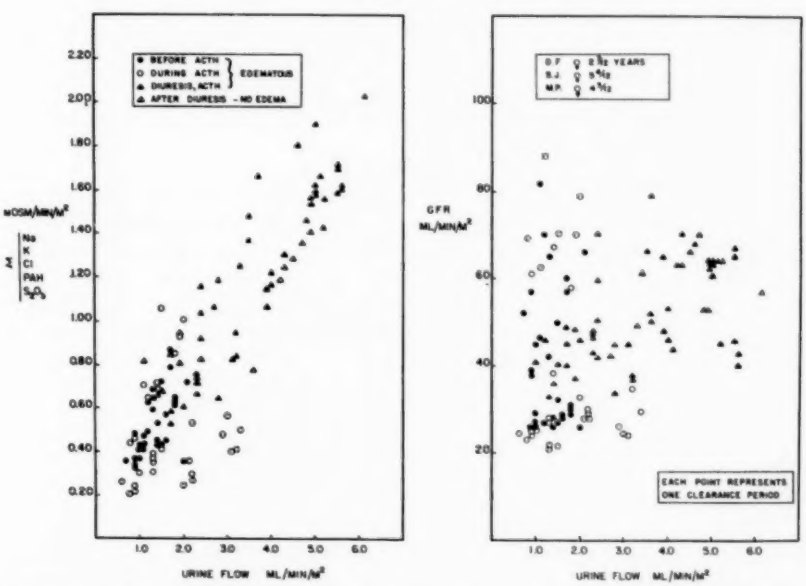

Fig. 9. Urine flow was related directly to solute excretion, but was not related to the absolute level of glomerular filtration. The largest solute excretions occasioned by infusion of nonreabsorbable anion loads (thiosulfate and para-aminohippurate) were observed at the onset of, and following, diuresis in 3 nephrotic children.

patient, M. P., an increase in renal plasma flow and glomerular filtration rate were noted during the paracentesis which required 40 minutes to complete. Very little change in intra-abdominal pressure occurred, however. Patient A. C. did not have the nephrotic syndrome or obvious renal disease; her massive ascites was due to congenital atresia of the bile ducts. Renal plasma flow appeared to be markedly reduced although the glomerular filtration rate was unchanged. Neither measurement was altered by paracentesis despite a remarkable fall of intra-abdominal pressure. In 2 patients, R. K. and M. M., electrolyte excretion was measured before and after paracentesis. In both instances, slight increases in sodium and potassium excretion were observed. Following paracentesis the changes were greater than could be accounted for by enhanced filtration of either sodium or potassium.

Plasma volume may influence glomerular filtration by alteration in renal blood flow and hydrostatic pressure. It has been suggested by Eder and co-workers[1] that plasma volume is diminished during active nephrotic edema and Bradley[30] has suggested that the nephrotic patient is in a state of quasi-dehydration because of diversion of plasma water to the interstitium. Plasma volume was measured by a modification of the technique of Chinard and Eder[55] using Evans blue dye (T1824) in 7 children prior to diuresis and these measurements were repeated 2 to 3 weeks later when the children were no longer edematous. The data are given in Table IV. Plasma protein concentrations and total circulating proteins increased in association with a slight increase in plasma volume and a moderate increase in glomerular filtration rates. The plasma volume increased significantly in 3 of 7 instances in which it was

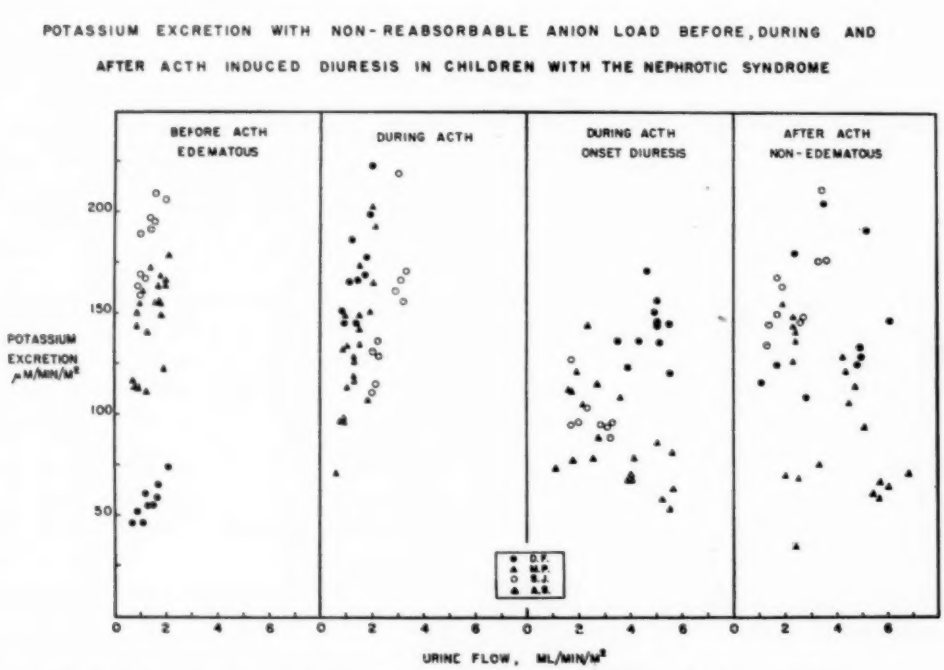

Fig. 10. Potassium excretion is independent of urine flow in nephrotic children receiving infusions of nonreabsorbable anions. The augmentation of potassium excretion with slight increments of urine flow before or during ACTH therapy, when edema was maximum, probably is more apparent than real, because of the very small change in urine flow caused by the limited osmotic diuresis. (See Figs. 8 and 9.)

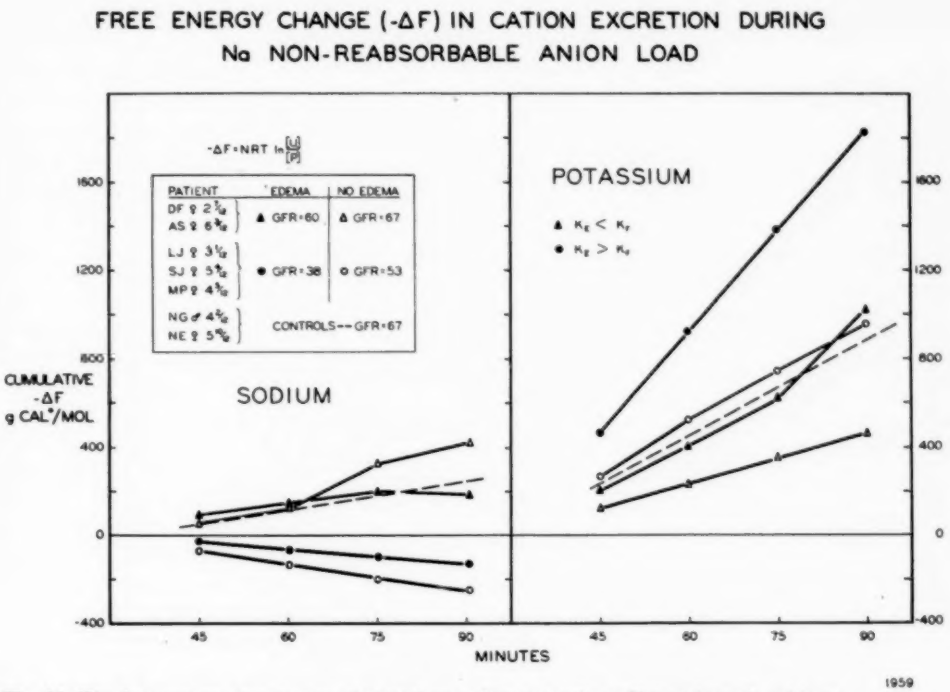

Fig. 11. The free energy change was calculated according to the basic Nernst formula, without subsequent modifications or refinements, which express osmotic work as some function of the osmolal ratios [P]/[U]. In this chart, the control values are averaged and shown as a broken line for comparison with the data from 5 nephrotic children. The nephrotic patients were studied during edema, and about 2 weeks later after diuresis. With diuresis, glomerular filtration rate rose appreciably in 3 of the children, but no significant change occurred in the $-\Delta F$ for sodium excretion. In contrast, a marked decrease in $-\Delta F$ for potassium excretion was observed after diuresis in the children who previously evidenced tubular K secretion ($K_E > K_F$).

measured, while slight increases were noted in 2 patients and decreased volumes were obtained in 2 other patients. The variations account for the large standard deviations and suggest that the mean change in plasma volume with diuresis may not be significant, at least when measurements are made at such a long interval. The glomerular filtration rate increased in 6 of the 7 patients. It decreased slightly in one child. Changes in the GFR of individual patients were significant, but the varying levels found in the group account for the large standard deviation of the average value. Additional studies of the effect of plasma volume expansion upon GFR, plasma volume, albumin filtration and excretion, and water diuresis were reported previously.[44]

2. Excretion of solutes and H_2O. The excretion of solutes and water is at least partially conditioned by the quantities of these substances provided by the intake and requiring excretion to maintain constancy of the body fluids. Variations in intake and modifications imposed by active treatment of the nephrotic syndrome make it difficult to compare performance of individual nephrotic patients or to compare nephrotic with normal subjects on the basis of unbiased renal capacity to excrete solutes and water. In order to obtain some degree of uniformity in challenge for patients with edema and for the same patients after diuresis, and to compare the responses of such individuals to those in nonedematous normal children, infusion of a solution of a sodium salt of a nonreabsorb-

able anion was chosen as a device to promote active tubular transport of solute and water under conditions which could be reasonably well standardized. A large amount of nonreabsorbable anion in the tubular luminal fluid requires chemically equivalent amounts of cation to maintain electrical neutrality of the fluid. The additional cation and anion in the luminal fluid limits the reabsorption of water. When the sodium salt of a nonreabsorbable anion is infused in a normal subject, the anion is promptly excreted with an almost equivalent amount of sodium.[56-58] The edematous nephrotic subject has some limita-

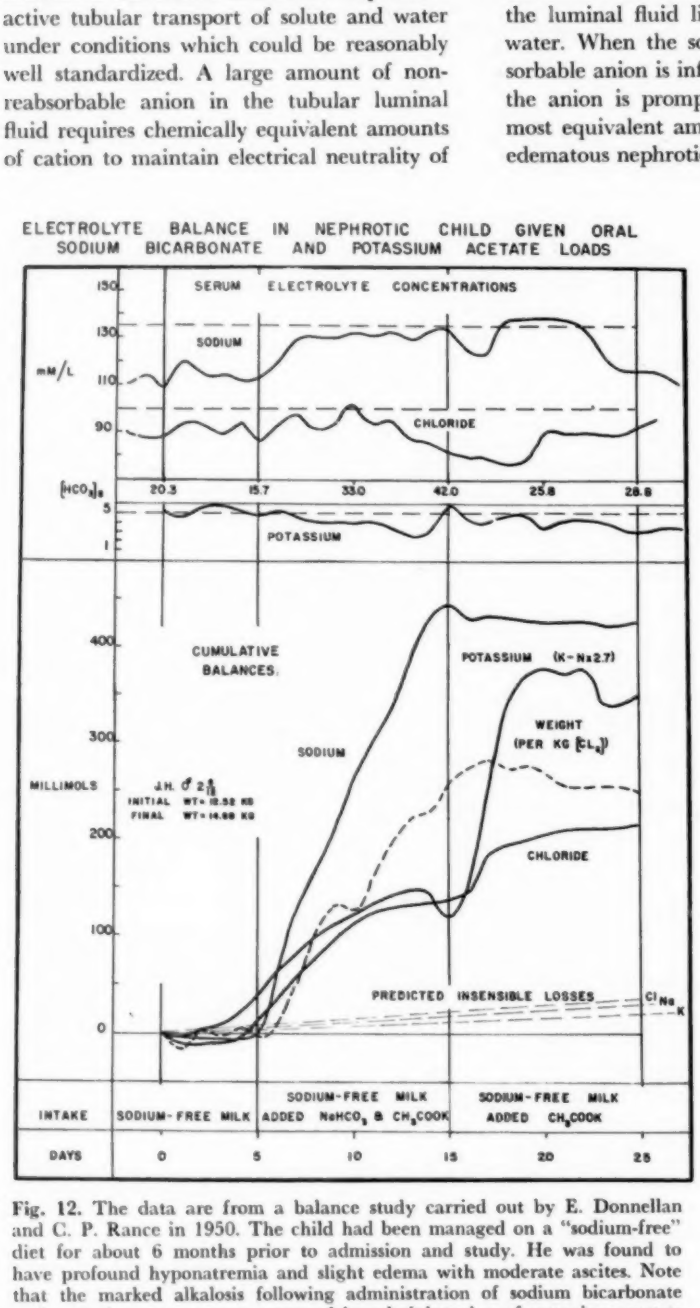

Fig. 12. The data are from a balance study carried out by E. Donnellan and C. P. Rance in 1950. The child had been managed on a "sodium-free" diet for about 6 months prior to admission and study. He was found to have profound hyponatremia and slight edema with moderate ascites. Note that the marked alkalosis following administration of sodium bicarbonate and potassium acetate was corrected by administration of potassium acetate with strikingly positive potassium balance (corrected for nitrogen); however, hyponatremia reappeared without significant change in either net sodium balance or body weight.

tion in rapid excretion of sodium, hence a nonreabsorbable anion load would require prompt secretion of hydrogen and potassium ions unless the stress situation could lead to mobilization of sodium. The ability of the kidney to provide the large quantities of hydrogen ion required during the rapid infusion of the loads under consideration might be somewhat limited; hence it might be anticipated that the excretion of potassium would be particularly augmented. Potassium excretion and sodium retention under circumstances of this sort have been previously reported.[59]

Sodium excretion and reabsorption subsequent to the infusion of a large sodium nonreabsorbable anion load must necessarily differ in edematous and control patients as a function of the volume of dilution and therefore of the portion of the infused load which achieves filtration during a short interval following infusion. It might be anticipated that the larger the volume of distribution of a given infused load, the lower the effective equilibrium concentration and hence the smaller the quantity of infused solute available for filtration and tubular challenge per unit time. The effect of nephrotic edema on the aliquot of infused sodium contributing to the sodium load, here called *the fractional filtered sodium load,* is indicated in Fig. 8. The volume of distribution of the infused sodium and the fraction of the load which was filtered were calculated.*

In the 3 patients illustrated in Fig. 8 it is clear that the quantity of sodium contributed by the infused load and available for filtration per unit time varies inversely with the volume of distribution of the sodium load. In the same patients following diuresis similar loads were confined to a much smaller volume of distribution, thereby providing a much larger quantity of solute for filtration per unit time, and hence a larger tubular load claiming excretion. The larger tubular load might be expected to result in a more profound osmotic diuresis. Such was the case as shown by the data in Fig. 9. As previously noted, sodium and potassium excretion were augmented by excretion of a nonreabsorbable anion and the excretion of these cations provided equivalence for major anion excretion, in this instance, thiosulfate, para-aminohippurate, and chloride. The greatest solute excretion for a load of a given size was observed during diuresis and thereafter when the patient was nonedematous. Osmotic diuresis is characterized by maximal urine flow and solute excretion. The solute concentration in the urine decreases to a level greater than, but approaching, that of the solute concentration in the body fluids. Although urine approaches isotonicity in maximal solute diuresis, a hypotonic urine $\left(\frac{U}{P} = < 1\right)$ is not observed.[60, 61] Nephrotic patients exhibit a typical solute diuresis. It should be noted, however, that, although solute excretion was proportional to urine flow, there was no relation between urine flow and glomerular filtration rate. A peculiar feature of solute diuresis in the nephrotic child is that potassium excretion is proportional to the augmented urine flows during the edematous phase but not during or after diuresis when the patient is nonedematous (Fig. 10). Such a lack of correlation between potassium excretion and the higher urine flows associated with osmotic diuresis is characteristic of the response of normal children to osmotic loads.[60] The excretion of potassium in edematous nephrotic children often exceeds the quantity

*1) $VDNa_1 = \frac{R\ Na_1 + (Na)_{T_1}}{[Na]_{ECW}}$ = volume of distribution of retained sodium, i.e., end of first infusion period.

2) $fFNa_1 = \frac{RNa_1}{VdNa_1} \times GFR$ = fraction of infused sodium which is filtered during Period 1.

3) $(Na)_{T_0} = VD_0 \times [Na]_{ECW}$ = initial Na content in its effective volume of distribution at time = 0 and $V_{DNa_0} = V_{DTh_{10}}$ = effective volume distribution of Na defined as equivalent to measured volume distribution of $Na_2\ S_2\ O_3$.

4) $(Na)_{T_1} = (Na)_{T_0} + RNa$ = retained Na/period (mM. infused − mM. excreted).

being simultaneously filtered and therefore must represent potassium secretion by the renal tubule to meet the obligatory anion excretion. The magnitude of such excretion has been described.[59]

Potassium secretion is more readily exhibited at reduced rates of glomerular filtration.[62, 63] This might be due to augmented high rates of sodium excretion with the load during a period of relatively reduced urine flow.[64] In the nephrotic subject secretion of potassium also is observed at normal levels of filtration, under conditions when control subjects similarly challenged and having similar filtration rates do not secrete potassium. This observation has suggested that some cellular transport mechanism, presumably energy dependent, is altered in the active nephrotic syndrome, irrespective of the state of sodium reabsorption. The nature of these presumed energy-dependent mechanisms is not clear, although the subject has intrigued renal physiologists for many years.

Several techniques for estimating the thermodynamic work, as a measure of the energy change associated with the excretion of particular solutes, have been suggested.[65-67] The limitations of such assessments with the data available to the renal physiologist, are quite obvious.[68-70] Yet the calculations serve to emphasize the significance of the gradients which must be established to achieve a final urinary concentration of a substance relative to its concentration in the plasma, e.g., the $\frac{U}{P}$ ratio. Described in "free energy" change terms, the observations on net renal work yield interesting, if speculative, inferences provided no emphasis is placed upon an "absolute" level of "free energy" change "characteristic" either of the normal or of the diseased kidney. The calculations may be somewhat more significant when applied to patients with renal disease having similar rates of glomerular filtration exhibiting varying patterns for excretion of sodium and potassium associated with the presence or lack of edema. Fig. 11 illustrates the cumulative "free-energy change" in cation excretion during loading with nonreabsorbable anion in two edematous nephrotic children before treatment with steroid and following diuresis when they were edema free some 3 weeks later. This is compared with changes in 2 control subjects of similar age and with comparable levels of glomerular filtration, defined by the broken line in Fig. 11, and in 3 edematous children with lower filtration rates, all subjected to similar challenges. The observations were made during infusion of sodium para-aminohippurate, following a previous injection of sodium thiosulfate, to provide a total sodium load averaging 155 mM. per square meter. Virtually all of the thiosulfate, given as a single injection, had been removed from the plasma within 90 minutes. Thereafter, a prime and subsequent sustaining load of para-aminohippurate was infused. Following a 30 minute equilibration period, observations made at 15 minute intervals were used as the basis for calculation of the "free-energy change." Patient D. F. failed to secrete potassium when edematous; A. S. secreted potassium only in the last 2 periods. L. J. had a normal glomerular filtration rate. The greatest potassium secretion was observed in Patients S. J. and M. P. who had the lowest filtration rates. After diuresis the excretion of potassium greatly exceeded that simultaneously filtered by S. J. although the glomerular filtration rate had doubled.

The observed slope defines the cumulative, exponential increment of "free energy" associated with excretion of sodium and potassium. In the edematous nephrotic subjects with normal GFR, the initial slopes defining the "free-energy change" seem to parallel those observed in the control subjects. In the last periods, however, there is a decrement in "free-energy change" associated with the excretion of sodium and an increment associated with the increased excretion of potassium.

Because of a greater $\frac{U}{P}$ ratio for potassium, the energy expenditure associated with transport against a gradient from tubular lumen to cell, and subsequently its secretion from tubular cell into the lumen to yield potassium for final excretion, according to

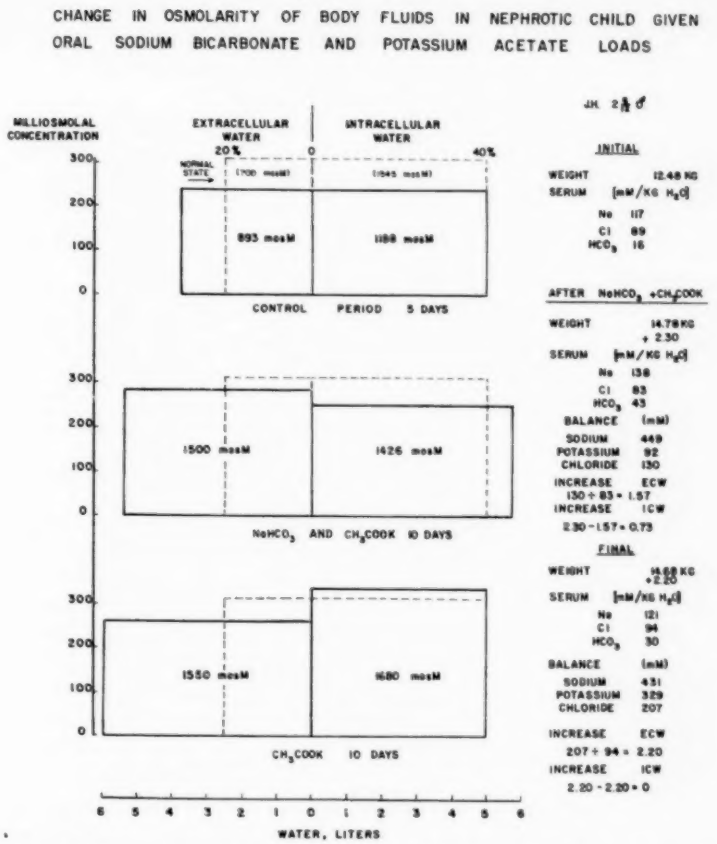

Fig. 13. Same patient illustrated in Fig. 12. The "shift" calculations indicate that the recurrent hyponatremia resulted from redistribution of body water with marked expansion of the extracellular phase. The apparent discrepancy between the osmolalities of the intracellular and extracellular fluids results from the assumption that all "intracellular" sodium and potassium are completely dissociated and the exclusion of the multivalent contribution of the anions to the calculation of intracellular osmolality.

current concept[64, 71] might account for the higher "free-energy change" associated with excretion of this ion. Consistent with this was the estimation that the greatest net "free-energy change" occurred in those subjects who excreted the largest amount of potassium. A striking diminution in the energy expenditure for potassium excretion is occasioned by diuresis, particularly in the patients who previously secreted potassium.

The excretion of water during osmotic diuresis induced in these various subjects averaged 3.1 ml. per 100 ml. glomerular filtrate in edematous nephrotic subjects with normal filtration rates, 5.9 ml. per 100 ml. glomerular filtrate in edematous subjects with diminished rates of glomerular filtration, and 7.3 ml. per 100 ml. glomerular filtrate in all nephrotic subjects after diuresis. These values should be compared with those of 3 control subjects undergoing a similar osmotic diuresis who excreted urine amounting to 3.9 ml. per 100 ml. of glomerular filtrate. Unfortunately, total solute concentrations were not measured directly at the time these studies were made and hence neither free water

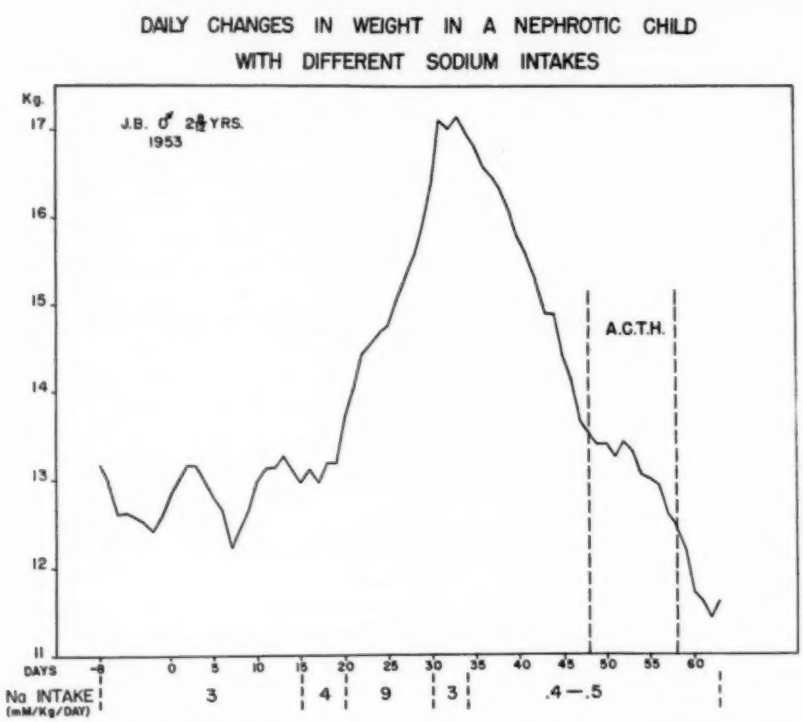

Fig. 14. This figure, as well as Figs. 15, 16, and 18, is taken from a study carried out by Dr. Gustavo Gordillo in our laboratories in 1953. Fig. 14 was published in a review of 150 cases of the nephrotic syndrome in children by Drs. Rafael Soto, A., Gustavo Gordillo, and associates: Bol. Med. Hosp. Infant. (Mex.) (English Edition) 1:8, 1960. It is reproduced with the permission of Dr. Gordillo and of the editors. Edema fluid accumulates, reflected in the sharp increment in body weight, when sodium intake exceeded 3 to 4 mM. per kilogram per day.

excretion nor reabsorption could be determined. However, the maximal osmotic $\frac{U}{P}$ ratio must have been slightly less than 2 judging from the sodium and potassium concentrations in the urine. Since urine flow in the controls averaged 3.9 ml. while in the edematous nephrotic patients it was circa 5 ml. per minute it is not possible to predict the maximal osmotic $\frac{U}{P}$ ratios.[72]

3. Electrolyte balances. Classical descriptions of the accumulation of edema fluid in the nephrotic syndrome with the use of balance techniques have been provided by Loeb and collaborators,[73] Magnus-Levy,[74] Blum and Van Caulaert,[75] Hoffman and Post,[76] Albright and Bauer,[77] and others. In general these descriptions have emphasized the retention of sodium and/or chloride as a characteristic feature of edema. Widal and Javal[78] suggested a specific renal defect in the excretion of chloride, while Magnus-Levy believed the defect lay in the excretion of sodium. Peters[79] favored a defect in chloride excretion, but stated that water was excreted with "quite as much or more difficulty than sodium." He suggested further that changes in the concentration of inorganic electrolytes in the extracellular fluids could not be directly connected with the absence or degree of edema.

In adult patients with nephrotic edema, because of the emphasis upon inability ef-

fectively to remove either sodium or chloride, salt restriction diets were suggested as the most practical means for control of the edema.[79, 80]

In general, young children with nephrotic edema do not tolerate the unpalatable diets resulting from stringent restriction of sodium and chloride. When occasionally a salt-free diet is consumed for a prolonged period by a compliant nephrotic child, gradual intracellular Na depletion may be intensified by intracellular K depletion since this "fixed" cation is removed in the urine to satisfy anionic equivalence during periods of maximal Na retention. If the diet is continued for a sufficiently long period without limitation of water, depletion of both extracellular and intracellular ions will lead to striking hypotonicity of the body fluids, commonly recognized as hyponatremia. Such an event is illustrated in Fig. 12. This patient was admitted for paracentesis early in 1950 because of accumulated edema and ascites despite having been on a stringently sodium-restricted diet ($<$ 1-2 mM. per day) for six months. Serum sodium concentration was found to be 110 mM. per liter of H_2O, and chloride concentrations were proportionately reduced. Balance measurements were made during repair of hyponatremia. For the first 5 days (control period) the intake was identical with that offered at home. Subsequently, 40 to 50 mM. of sodium bicarbonate and 20 to 30 mM. potassium acetate daily were added to the diet. Cumulative balances over the next 10 days indicated a retention of sodium approximating 450 mM., four times that of the simultaneously observed chloride balance. In the chart, the increment of weight is plotted on an ordinate constructed so that 1 kilogram may be related to a volume of 1 L. defined by the concentration of chloride in the extracellular fluid. The retention of sodium was considerably in excess of the gain in body weight, indicating that about one half of the retained sodium was not in the extracellular phase. When both sodium bicarbonate and potassium acetate were given, despite positive balances of potassium, alkalosis developed, and serum potassium concentration fell to dangerous levels. Metabolic alkalosis in association with hypokalemia and sodium loading is thought to characterize cellular potassium depletion.[81] In the following 10 day period, 60 to 75 mM. potassium acetate was the only supplement. In this instance, repair of alkalosis was effected by administration of a potassium salt with a bicarbonate equivalent, and was characterized by remarkable retention of potassium in excess of nitrogen. Sufficient potassium was retained to increase the calculated intracellular concentration of the ion by 55 to 60 mM. per liter, thus indicating the very large K deficit engendered by the protracted Na-depleted diet.

With the initial potassium retention, sodium concentrations increased to normal levels as described later by Laragh,[82] but the increased concentration was not sustained and hyponatremia recurred. The recurrent hyponatremia must in this instance have been the result of a redistribution of total body water. Contraction of the intracellular phase and expansion of the extracellular fluids must have occurred since significant loss of Na was not observed, and intracellular K concentrations were increasing while total body weight decreased. The calculated changes in osmolarity and distribution of the body fluids in this child are indicated in Fig. 13. The volumes of extracellular and intracellular fluids are defined on the abscissa and the solute concentrations of these fluids on the ordinate. The broken line indicates the predicted normal state, and the product of solute concentration and volume determines the solute content of the phase according to the technique of Darrow and Yannet.[83] The solute concentration was assumed to be equivalent to twice the sum of sodium plus potassium concentrations. It was assumed that the modest edema present during the control period was essentially extracellular. The change in water and solute content of the intracellular phase shown in the intermediate and final portions of the diagram were derived from the usual Darrow "arithmetic"[84] based on the assumed

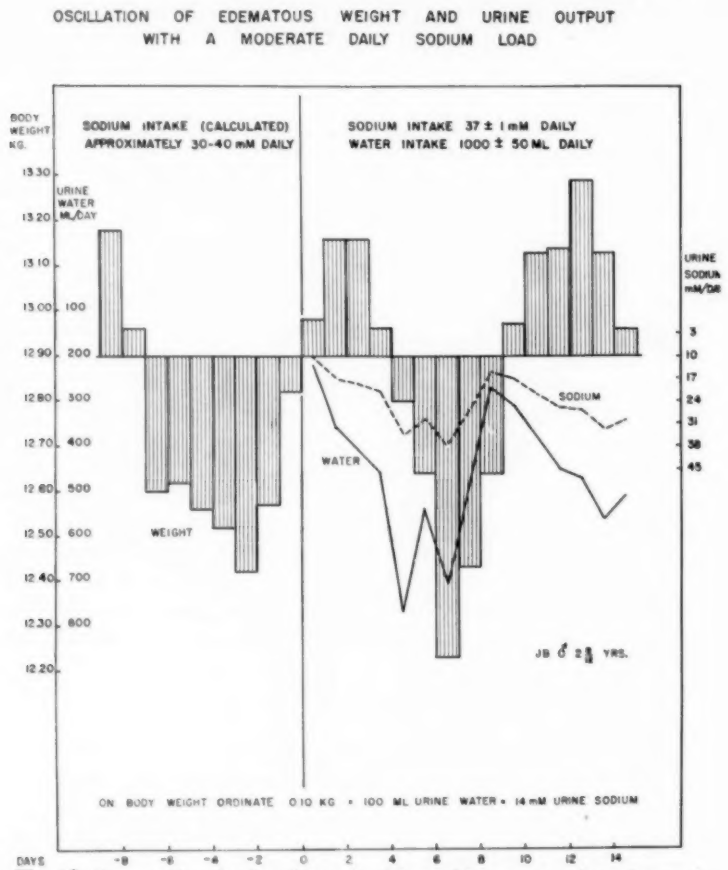
OSCILLATION OF EDEMATOUS WEIGHT AND URINE OUTPUT
WITH A MODERATE DAILY SODIUM LOAD
BODY WEIGHT KG.
SODIUM INTAKE (CALCULATED) APPROXIMATELY 30-40 mM DAILY
SODIUM INTAKE 37 ± 1 mM DAILY
WATER INTAKE 1000 ± 50 ML DAILY
URINE WATER ML./DAY
URINE SODIUM mM/DAY
SODIUM
WATER
WEIGHT
JB ♂ 2½ YRS.
ON BODY WEIGHT ORDINATE 0.10 KG. = 100 ML URINE WATER = 14 mM URINE SODIUM
DAYS

Fig. 15. On a sodium intake of 3 to 4 mM. per kilogram per day, changes in body weight were preceded by opposite variations in the excretion of water and sodium. The oscillatory adjustment to a constant, moderate intake of sodium is evident.

extracellular position of chloride. No consideration was given to possible changes in valence or binding capacities of intracellular anions. It is apparent that during the initial repair period when sodium bicarbonate and potassium acetate were added to the Na-free milk intake, considerable expansion of both intra- and especially extracellular phases occurred with some improvement in solute concentrations. With the subsequent administration of potassium acetate only, contraction of intracellular volume restored intracellular concentrations to normal.

In the final period of study the increment of water in the extracellular phase was equivalent to its decrement in the intracellular phase; thus the hypotonicity* observed at the end of the balance presumably represented a mobilization of cellular fluid. If hyponatremia is associated with an increase in intracellular water with consequent dilution of depleted intracellular cations and

*It is interesting that this patient with hyponatremia manifested an extraordinary degree of plasma turbidity and hyperlipemia which cleared noticeably following intravenous albumin infusion. The measurements were made by Dr. Alan Crocker on Feb. 5, 1950, and were as follows: total lipids 9,700 mg. per cent, neutral fat 7,260 mg. per cent, total cholesterol 1,072 mg. per cent, phospholipids 968 mg. per cent, turbidity 223 units. Normal values are: for total lipids 400 to 800, neutral fat 0 to 150, total cholesterol 150 to 260, phospholipids 150 to 250 mg. per cent, and turbidity 2 to 4 units.

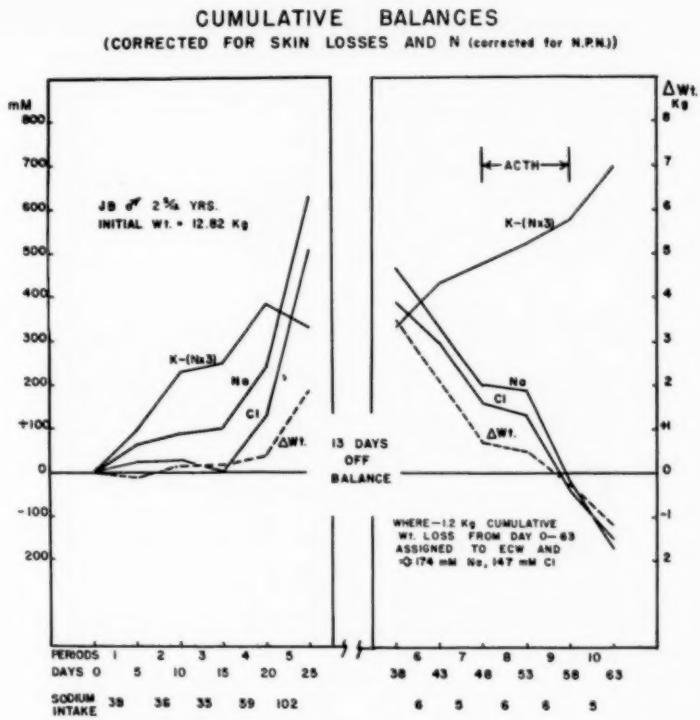
CUMULATIVE BALANCES
(CORRECTED FOR SKIN LOSSES AND N (corrected for N.P.N.))
mM
ΔWt. Kg
JB ♂ 2 ¾ YRS.
INITIAL WT. = 12.82 Kg
ACTH
K-(Nx3)
Na
Cl
ΔWt.
13 DAYS OFF BALANCE
WHERE—1.2 Kg. CUMULATIVE WT. LOSS FROM DAY 0—63 ASSIGNED TO ECW AND =Q 174 mM Na, 147 mM Cl
PERIODS 1 2 3 4 5 6 7 8 9 10
DAYS 0 5 10 15 20 25 38 43 48 53 58 63
SODIUM INTAKE 39 36 35 59 102 6 5 6 6 5

Fig. 16. The ordinate is so constructed that 100 mM. (the approximate chloride concentration of the extracellular fluid) is equivalent to 1 kilogram of body weight. Therefore, if all chloride is retained in the free extracellular fluid, and the increment of body weight with edema represents expansion of the extracellular fluid, cumulative chloride balance should be superimposed on the Δ weight balance. The same would be true, in reverse, for diuresis. It is evident that both retention and loss of Na and Cl are greater than expectation from the above assumption; therefore accumulation of these ions with edema and their loss with diuresis indicate deposition in some phase or phases other than the freely diffusing extracellular fluid.

substrates, it is possible that the metabolic activity of the cell would be reduced and sodium diffusing into it would accumulate, failure of extrusion thus leading to a collapse of the sodium gradient and swelling of the cell.[85]

In addition to the above study, the frequent occurrence of hyponatremia complicating steroid therapy during sodium-restricted diets in children with the nephrotic syndrome[41] suggests that protracted and strict dietary Na restriction must be used with great care, if at all, in the management of children with nephrotic edema. In contrast, dietary Na restriction seems more efficacious in control of edema occurring in the adult with the nephrotic syndrome.

If stringent sodium restriction is not desirable, then the amount of sodium which may be provided in the diet without causing excessive edema remains to be determined. Fig. 14 illustrates change in body weight of an edematous nephrotic child during varying sodium intakes. It will be noted that a cyclic variation in body weight was observed with sodium intakes ranging between 3 and 4 mM. per kilogram per day. This intake is equivalent to the "load" provided in the classical study of Loeb, Atchley, and associates.[73] When an excessive sodium intake of

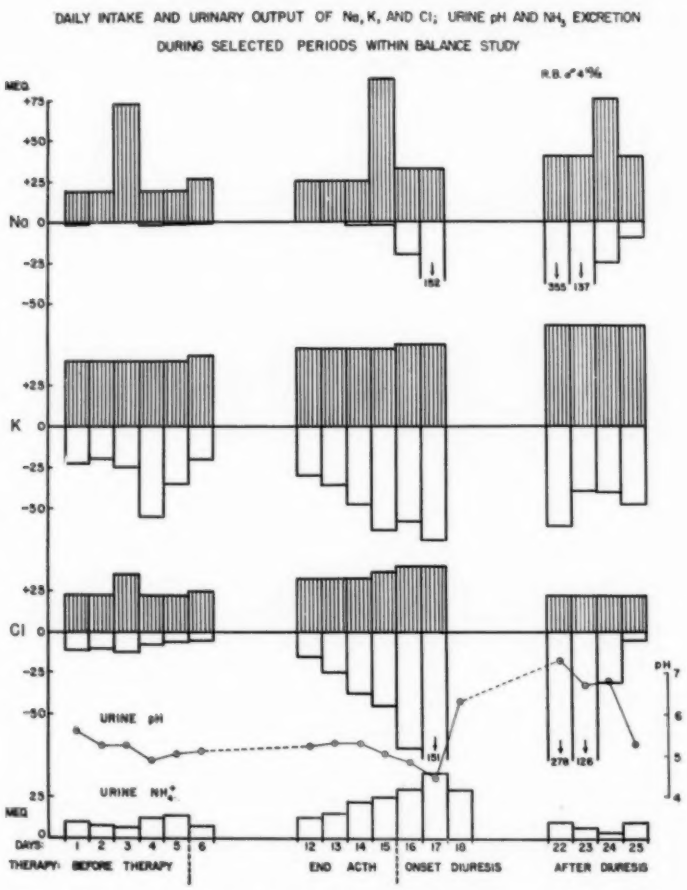

Fig. 17. The data are from a balance study performed by J. James in 1953. Note the increase in urinary sodium, chloride, and ammonium excretions, and the appearance of an alkaline urine, with diuresis. The increased excretion of potassium on days 4 and 15 to 17 probably was related to large sodium loads on days 3 and 15 of the study. After diuresis, a similar sodium load did not alter potassium balance.

9 mM. per kilogram per day was given, edema rapidly increased, and body weight almost doubled. Following reduction of intake to a moderate level of 3 mM. per kilogram per day further accumulation of edema ceased and diuresis was initiated. This early diuresis was not recognized, and the dietary intake of sodium was reduced (according to plan) to 0.4 to 0.5 mM. per kilogram per day. Diuresis progressed and was not significantly inhibited by administration of ACTH. A detail relating the oscillation of edematous weight to urinary volume and sodium excretion during the period of moderate sodium intake is illustrated in Fig. 15. It is clear that the periodic oscillation in body weight above and below a "steady state" weight characterizing a moderate sodium intake, even in a nephrotic patient, is reflected in the renal excretion of water and sodium as previously described.[73, 86, 87] Excessive intake resulted in a marked accumulation of these ions. The accumulation of sodium and chloride was considerably greater than could

be accounted for by the expansion of body water even if it was assumed that all of the retained sodium and chloride were contained at a concentration equivalent to that of the extracellular fluid and therefore that all weight gain represented extracellular fluid (Fig. 16).

By the twenty fifth day of the balance study, the apparent cumulative retention of sodium was about twice, and of chloride about two and one-half times, that expected from gain in body weight, presumably edema fluid. Potassium balance, corrected for that which might be deposited with nitrogen, also was positive.

Diuresis of nephrotic edema was charac-

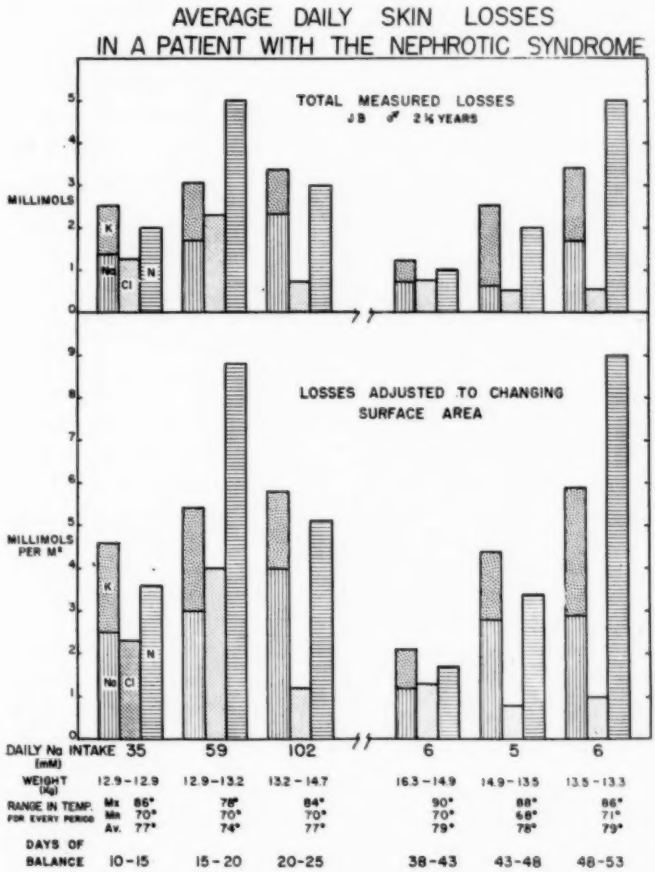
AVERAGE DAILY SKIN LOSSES
IN A PATIENT WITH THE NEPHROTIC SYNDROME
TOTAL MEASURED LOSSES
J B ♂ 2¼ YEARS
MILLIMOLS
K
Na
Cl
N
LOSSES ADJUSTED TO CHANGING SURFACE AREA
MILLIMOLS PER M²
DAILY Na INTAKE (mM) 35 59 102 6 5 6
WEIGHT (Kg) 12.9-12.9 12.9-13.2 13.2-14.7 16.3-14.9 14.9-13.5 13.5-13.3
RANGE IN TEMP. FOR EVERY PERIOD Mx 86° 78° 84° 90° 88° 86°
Mn 70° 70° 70° 70° 68° 71°
Av. 77° 74° 77° 79° 78° 79°
DAYS OF BALANCE 10-15 15-20 20-25 38-43 43-48 48-53

Fig. 18. The patient was carefully washed with distilled water at the start of a period, and clothed in a single distilled-water-washed cotton garment. After 5 days, the garment was removed and the patient again washed with distilled water. Washings were saved. The skin secretions were eluted from the cotton shirt and were pooled with the bath washings. The pooled fluid was concentrated to convenient volume by evaporation and analyzed.

There appears to be some correlation between the skin losses of sodium and increasing sodium intakes during each of the 5 day periods of increasing intake from 35 to 102 mM. per day. Skin losses of sodium and potassium, but not of chloride, increased during diuresis (days 38 to 48), despite a low-sodium intake. Nitrogen losses were variable.

terized by removal of sodium and chloride in a large urine volume at a concentration only slightly greater than that obtaining in extracellular fluid. In contrast to the accumulation of edema fluid, diuresis represented removal of a fluid simulating the extracellular fluid plus an additional small amount of sodium and chloride, presumably from other reservoirs. It is obvious that loss of edema fluid by diuresis does not necessarily have the reverse metabolic implications to accumulation of edema fluid. Typical patterns of urinary composition during selected periods relating to diuresis observed in another balance study (on a $4\frac{10}{12}$-year-old male) are indicated in Fig. 17. It will be noted that, with the onset of diuresis, urinary pH and ammonium excretion rose simultaneously with the increase in sodium and chloride excretion. As diuresis terminated, approximately 8 days after its onset, the urine contained normal quantities of sodium and chloride, although potassium excretion remained relatively large and provided the principal cation of the urine.

Since the measured retentions of Na, Cl, and K were in excess of amounts readily assigned to either extracellular or intracellular fluids, it seemed possible that some error in the balance technique had occurred; for example, it might be presumed that the skin losses of electrolytes could account for much of the discrepancy. In this instance, however, the average daily skin losses were measured (see Fig. 18) and the balances corrected accordingly. It is interesting that the skin losses of sodium in this patient appear to be proportional to intake during the periods of increasing intake and were decreased during diuresis and periods of low sodium intake. The large and variable excretions of nitrogen from the skin appeared to be independent of intake.

The calculations on the following page derived from this study illustrate some of the difficulties in interpretation of sodium balance measurements during nonsteady states.

Obviously, neither calculated expansion of the extracellular water volume, nor generalized increase in body content of sodium per kilogram of body weight yields estimates which approximate the observed changes. Nor do such calculations provide reasonable interpretations of fluid compartment changes. Apparently, more millimols of sodium were retained than could be accounted for by this type of arithmetic.

One possible solution for this dilemma of the "missing millimol" was offered by Blum[75] and by Fanconi,[88] who suggested that skin may retain chloride (and presumably sodium) in greater concentration than other portions of the extracellular phase. These suggestions were consistent with observations of Klose on the distribution of electrolytes in entire organ systems of 2 edematous infants as determined by direct analyses.[89] Assuming that the tissue distribution of retained electrolyte observed in our balance on this edematous child was proportional to that found by Klose it should be possible to estimate the quantity of water and electrolyte retained in connective tissues[90] and compare these quantities with those contained in edematous skin and skeleton, and, thereby, to "correct" the crude balance. The "corrected" balance would indicate the quantity of sodium, potassium, and chloride which might properly be assigned to muscle and organs and subjected to calculations of labile "cellular" distributions according to Darrow (Table V).

The calculations of Table V indicate that more than half the retained sodium and chloride were deposited in skin whereas the great mass of retained potassium was not and must have been in muscle, viscera, or skeleton. According to this calculation it would appear that the extraordinarily large retention of sodium would not have appreciably changed the concentration of sodium in metabolically important cellular tissues such as muscle or viscera. Obviously these calculations represent only crude, first order approximations until direct measurements of the composition of skin and skeleton from edematous nephrotic children, as well as from nonedematous children, provide a more suitable basis for proper correction of balance measurements, and probable

Estimated change in body composition for observed retention of sodium:

Observed: Initial [Na] of ECW = 131 mM. per liter; final = 143 mM. per liter
Body weight initial = 12.82 kilograms
Final = 13.75, Δ + 1.93 kilograms
Na balance = +626 mM.

1. Assigning all retention and weight gain to ECW

Initial ECW = 12.8 × 0.3 = 3.74
Final ECW = 3.74 + 1.93 = 5.67
Initial ECW (Na) = 3.74 × 131 = 490 mM.
Then Final ECW (Na) = 5.67 × 143 = 810 mM.

A. Theoretical retention = 810 − 490 = 320 mM.
but observed retention = 626 mM.

B. Or estimated increase ECW = 490 mM. + 626 mM. = 1,116 mM. Na

and $\frac{1116}{143}$ = 7.8 L. ECW

But Δ weight = 1.93 kilograms

C. Then shift of ICW to ECW

7.8 (final calculated)
−5.6

2.2 L. from ICW to ECW

But initial ICW = 12.8 × 0.4 = 5.12 and 5.1 − 2.2 = 2.9 L.

2. Estimated increase $[Na]_{ECW}$ and body weight for Na retention

Assuming initial (Na) = 42 mM. per kilogram
Initial body content Na = 42 × 12.8 = 537
Observed retention = 626

Final content Na = 1,163

Per kilogram = $\frac{1,163}{13.75}$ = 85 mM. per kilogram

Estimated final [Na]

$\frac{85}{42} \times 131 = 265$ mM. per liter

Observed = 143 mM. per liter
Estimated increase in body weight
Retention = 626
Initial body content = 42 mM. per kilogram

Estimated final weight increase = $\frac{626}{85}$ = 7.4 kilograms

Observed = 1.9 kilograms

solution of the problem of the "missing millimol." Some preliminary observations on samples of skin removed at operation from "normal" children 1 to 4 years of age indicated that 3 to 4 mM. per 100 Gm. of dry fat-free solids of the sodium and chloride found in the skin are in "excess" of amounts distributed in both extra- and intracellular fluids, and therefore must be affixed to collagen. This represented approximately 12 per cent of the estimated total skin content of Na and Cl in these normal children (Table VI).

The calculated potassium concentration of muscle and organs after diuresis apparently increased by about 50 mM. per liter ICW (Table V). The increment in potassium concentration suggests an antecedent deficit of this ion previously acquired during edema, and further emphasizes the unusual lability

Table V. Correction of balance measurements for skin and skeletal deposition of Na and Cl (J. B., $2\frac{8}{12}$ years old, nonedematous weight 11.61 kilograms, edematous weight 13.75 kilograms)

A

	Nonedematous				*Edematous*			
	Body weight	*Na*	*K* (*mM./Kg. FFT*)	*Cl*	*Body weight*	*Na*	*K* (*mM./Kg. FFT*)	*Cl*
Skin	16%*	101	35.8	37.2	30%	94	14	67
	1.86 Kg.	188	67	69	5.50	517	77	368
Skeleton	17%*	72	14.5	49	17%	72	14.5	49
	1.92 Kg.	142	29	97	2.34	169	34	115

B

Increment organ contents with edema

	H_2O (*Kg.*)	*Na* (*mM.*)	*K* (*mM.*)	*Cl* (*mM.*)
Skin	3.64	329	10	299
Skeleton	0.42	27	5	18
Total	4.06	356	15	317

C

Balance corrected for skin and skeleton

	Na	*K*	*Cl*
Balance	626	333	511
-Skin and skeleton	356	15	317
Residual in muscle and organs	+270	318	194

D

"Cellular" distribution from balance (After Darrow)

25 days Δ weight = +1.93 Kg.

	ECW	*ICW*	*Na_e*	*Na_i*	*K_e*	*K_i*
Initial	3.20	5.70	420	-	16	-
Final	+5.35	-3.42	+803	-177	+26	+307

E

Water distribution corrected for skin and skeleton

$$\Delta \text{ ECWf} = +5.35 - 4.06 = +1.29$$
$$\text{ECWf} = 3.20 + 1.29 = 4.49$$
$$\Delta \text{ ICW} = \Delta \text{ WT.} - \Delta \text{ ECW} = 1.93 - 1.29 = 0.64$$
$$\text{ICWf} = 5.70 + 0.64 = 6.34$$

F

Sodium distribution corrected for skin and skeleton

$$(\text{Na})_e \text{ initial} = 3.20 \times 131 \quad 420 \text{ mM.}$$
$$(\text{Na})_e \text{ final} = 4.49 \times 143 \quad 642 \text{ mM.}$$
$$\Delta\,(\text{Na})_e = +222 \text{ mM.}$$
$$(\text{Na})_i = 270 - 222 = +48$$
$$[\text{Na}]_i = \frac{48}{6.34} = +7 \text{ mM./L.}$$

G

Potassium distribution corrected for skin and skeleton

$$(\text{K})_e \text{ initial} = 3.20 \times 5.0 = 16 \text{ mM.}$$
$$(\text{K})_e \text{ final} = 4.49 \times 4.9 = 22 \text{ mM.}$$
$$\Delta\,(\text{K})_i \text{ final} = +318 - 6 = 312 \text{ mM.}$$
$$[\text{K}]_i \text{ final} = \frac{312}{6.34} = +49 \text{ mM./L.}$$

*Relative organ weights as per cent of body weight (BW) from Klose.[89] Composition of skin and skeleton from Shohl.[90]

Table VI. "Excess"* sodium and chloride in biopsies of skin† of 7 "normal" children $1\frac{3}{12}$ to $4\frac{2}{12}$ years of age

	H_2O (Gm.)	Na (mM.)	Cl (mM.)	K (mM.)	[ICW]ss		[ECW]		$(Na)_{ex}$ (mM./100 Gm. DFFS)	$(Cl)_{ex}$ (mM./100 Gm. DFFS)
					Na	K	Na	Cl		
m	267.9	34.3	29.0	8.0	22.6	159.3	142.1	115.4	4.1	3.6
±S.E.	24.0	1.9	0.9	0.5	3.0	3.4	1.4	1.4	1.7	2.0

*For calculation, see Table IX.

†Skin plus subcutaneous tissue.

Table VII. Muscle composition in a patient with a congenital nephrotic syndrome

A. Per 100 Gm. DFFS

		H_2O (Gm.)	Na (mM.)	K (mM.)	Cl (mM.)	P_T (mM.)	P'_i (mM.)	P_e (mM.)	Pyr. (μM)	α-K (μM)
J. A. ♂		504	29	33	22	16	8	8	38	889
Controls	(14)	369	20	38	15	27	14	13	169	75
	SE±	4	1	0.5	0.4	—	—	—	—	—

B. Intracellular concentration

	H_2O (Gm.)	Na (mM./L.)	K (mM./L.)	P_T (mM./L.)	P'_i (mM./L.)	P_e (mM./L.)	Pyr. (μM/L.)	α-K (μM/L.)
J. A.	334	8	97	49	24	25	115	2662
Controls	236	15	157	74	43	31	716	322
SE±	5	2	3	—	—	—	—	—

C. Intracellular content/mol pyruvate

	Na	K	P_T	P'_i	P_e	α-K
J. A.	73	841	422	208	214	23
Controls	21	216	160	83	77	0.4

Table VIII. Distribution of edema in 18 nephrotic rats versus pair-fed controls

	Pair-fed control (Gm.)	*Edematous nephrotic (Gm.)*
A. Body weight	108 ± 2.6	143.5 ± 5.6
B. Ascites	0	25.9 ± 2.9
C. Fat	3.9 ± 0.5	2.2 ± 0.3
D. FF Body weight − ascites	104	115.4
E. Skin H_2O	9.3 ± 0.1	15.7 ± 1.8
F. Carcass H_2O	49.3 ± 2.2*	50.0 ± 1.5†
G. Tissue edema ($D_{control} - D_{edema}$)	—	11.4

Distribution of edema fluid

Edema = 37.3 Gm. = 26% Body weight
Ascites = 69.4%
Skin = 17.2%
Viscera = 11.4%
Carcass = 2.0%

Distribution of tissue edema

(Edema − ascites = 11.4 Gm.)
Skin = 56.1%
Viscera = 37.3%
Carcass = 6.6%

*Nine carcasses.

†Fifteen carcasses.

of K in the nephrotic child. This inference is supported by a few direct analyses of muscle composition of nephrotic children.[91] The subjects studied by Fox previously had been treated with oral supplements of $NaHCO_3$ + KCOOH; however, the composition of muscle obtained at biopsy from a 1½-year-old male infant with a congenital nephrotic syndrome on a moderate (2 to 3 mM. per kilogram per day) Na intake (Table

VII) also is consistent with this thesis. Compared to controls, there was a significant reduction of K as well as of organic and "apparent" inorganic phosphate in the cell. Intracellular electrolyte concentrations were markedly reduced, primarily because of dilution. That some cellular metabolic sequence had been interrupted was suggested by the accumulation of alpha-ketoglutarate and the striking relative excess of electrolyte per mol of pyruvate. Obviously further studies of cell composition in nephrotic children are required.

4. Metabolism of ions. The distribution of water and ions in various tissues and the changes in cell composition and function can only be estimated in the nephrotic patient. Obviously, many features of the syndrome might be examined in greater detail and the validity of some clinical inferences appraised by study of the experimentally produced disease. To this end, the nephrotic syndrome was induced in weanling male Sprague-Dawley strain rats by subcutaneous injection of 6-dimethylamino purine riboside (amino-nucleoside).[92]

Muscle, skin,* organs, and residual carcass were analyzed separately. Bone and tendon were included with the carcass analyses. The various tissues were weighed, frozen in liquid nitrogen, and pulverized in a specially designed large "rat smasher" in a cold room at 0° C. The frozen, pulverized tissues were ground further in a cold mortar and pestle and transferred to weighing bottles. Weights of the processed tissues indicated total losses of less than 2 per cent in the procedure. Fat was extracted from tissues with a petroleum and ethyl ether mixture. The dry, fat-free solids (DFFS) were extracted either by digestion in distilled water in a carefully sealed tube by autoclaving at 20 pounds pressure and 120° C. temperature for 4 hours, or by "wet ashing" with the use of 0.75 N HNO_3.

Analytical results for edema fluid from 18 nephrotic rats are shown in Table VIII. Most of the edema accumulated as free ascitic fluid with the composition of extracellular fluid; however, excluding ascites, approximately 56 per cent of the tissue edema was accounted for in skin, 37 per cent was distributed throughout the viscera, and the remaining 7 per cent in the carcass. Skin appears to be the major *tissue* reservoir for edema fluid in nephrotic rats.

Estimates of the distribution of ions in skin have been reviewed by Manery.[94] She emphasized that the usual technique of calculation based upon either the extracellular distribution of chloride or the volume of distribution of sodium gave inconceivable results for the composition of cell fluid in the skin, since the potassium concentration of skin cells would be well over 200 mM. per liter of intracellular water. Other techniques of calculation have been employed[93] which improve the estimates to some degree. If, however, simultaneous assay of the concentration of sodium and potassium within a typical cell such as muscle is available, it is possible to calculate the distribution of ions in skin based upon the assumption that the composition of viable skin cells is similar to that of muscle cells (see Table IX). The intracellular volume is estimated from K content and concentration and the remaining water assigned to the extracellular phase. Given the intracellular concentrations of Na (from muscle) and the extracellular concentrations of sodium and chloride, the quantity of these ions contained within the extracellular phase then can be allocated. Such calculations indicate that skin contains appreciable quantities of sodium and chloride in excess of that which can be assigned to

*The initial studies of edematous rat skin indicated that the location of the biopsy and quantity of skin influenced the data. To overcome this problem total skin was analyzed. Rats were anesthetized with Evipal or Nembutal anesthesia and depilated by the technique of Walser and Bodenlos.[95] A small incision was made in the abdomen of edematous rats and a plastic catheter attached to an aspirating device was introduced to remove all ascitic fluid. The abdomen was then opened, the animal was exsanguinated by aspiration from the abdominal aorta, viscera were removed, and the entire skin was stripped from the carcass in essentially the same way one removes a glove from the hand. Entire skins could be removed without loss of fluid. The water contained in the skin was present as a gel and resembled Wharton's jelly in appearance and consistency. When placed in a weighing bottle at room temperature such gels soon liquified and the epthelial tissue rapidly became submerged in the fluid previously comprising the gel.

either the intracellular or extracellular fluid. The nonextracellular chloride and excess sodium may be fixed by an electrostatic bond to charged polar end-groups on the collagen fibril. As the collagen fibrils are separated by hydrophilic swelling their parallel orientation, previously maintained by the interdigitation of oppositely charged polar end-groups, becomes distorted, uncovering the charges. According to the thesis advocated by Bear,[96] these uncovered charges may be satisfied by acquisition of cations and anions abundantly present in the surrounding medium. At the pH of the body fluids, sodium and chloride statistically would provide the largest quantity of these ions. Since the number of anionic sites available for saturation would be equal to the number of cationic sites, an amount of chloride essentially equivalent to that of sodium would be loosely "bound," and, while bound, presumably would be osmotically inactive as demonstrated for Na, K, and Ca on chondroitin sulfate polyanion.[97] The chloride: sodium ratio of skin, therefore, would be relatively greater than the chloride:sodium ratio of the extracellular fluid.

Comparisons of electrolyte increments in nephrotic rats and their pair-fed controls based on total body analysis with those calculated from balance measurements reveal an apparent retention of potassium by the balance technique but a net loss of potassium according to direct analysis of total carcass.[98] Table X indicates that during the development of the nephrotic syndrome in the grow-

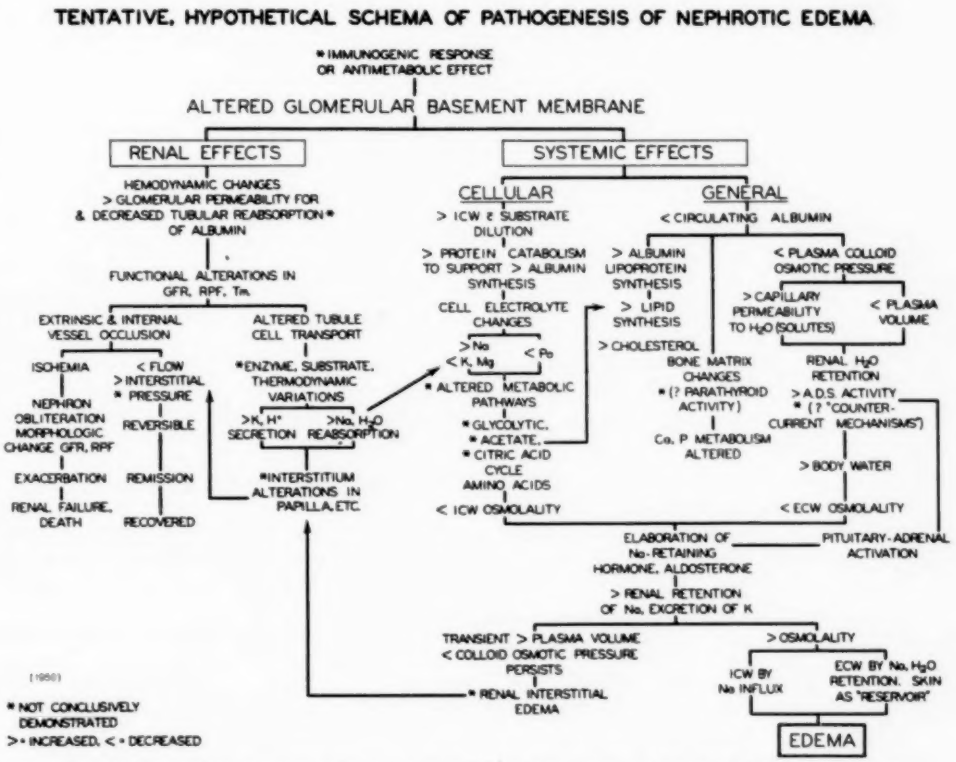

Fig. 19. Since this chart was constructed, evidence has appeared substantiating alteration of pathways for glycolytic, citric acid cycle and acetate metabolism. The hypothesis is described in the text.

ing rat the nephrotic animal gained less dry fat-free solids and potassium and a disproportionately greater quantity of water than did the pair-fed control. Diuresis was induced in the nephrotic rats over a 5 day period by cessation of aminonucleoside ingestion and incorporation of a carbonic anhydrase inhibitor in a sodium-free diet.

A further feature of total body analysis in rats may be directly applicable to clinical experience. The apparent wasting of the tissues of nephrotic children, frequently noted after diuresis following a protracted siege of edema, has suggested that tissue catabolism may be a constant feature of the nephrotic syndrome which contributes to the dynamic equilibrium of the plasma protein pool, in turn depleted by both proteinuria and increased catabolism.[21] Although balance studies suggest an antecedent depletion of body potassium, studies with exchangeable K_{42} did not.[99] The data in Table X illustrate a diminished gain in dry fat-free solids by the growing, edematous nephrotic rats relative to their pair-fed controls. With diuresis there was reduction in total potassium and a

Table IX. "Excess" sodium and chloride in skin of nephrotic rats

A. Content (per 100 Gm. NC DFFS)

	No.	*H_2O (Gm.)*	*Na (mM.)*	*K (mM.)*	*Cl (mM.)*	*Collagen (/100 Gm. DFFS) (Gm.)*
Control	6	424.2	43.0	33.1	37.5	36.76
	±S.E.	7.4	1.4	1.9	1.6	0.76
Nephrotic	6	759.8	93.2	30.8	77.9	37.24
	±S.E.	86.0	8.3	0.9	5.8	1.87
Control + NaCl load	12	380.3	40.1	27.4	33.0	33.77
	±S.E.	10.9	1.4	0.6	0.8	1.11
Nephrotic + NaCl load	12	800.0	113.9	27.1	92.2	35.27
	±S.E.	90.1	12.6	0.9	10.5	1.00

*B. "Excess" Na and Cl**

	$[ICW]_m$		$[ECW]$		H_2O		Cl			Na		
	K (mM./L.)	*Na (mM./L.)*	*Na (mM./L.)*	*Cl (mM./L.)*	*ICW (ml.)*	*ECW (ml.)*	*$(Cl)_{ECW}$ (mM.)*	*Excess (mM.)*	*(%)*	*$(Na)_{ECW}$ (mM.)*	*Excess (mM.)*	*(%)*
C	161.7	20.1	143.3	125.8	198.5	225.7	28.4	9.1	24.2	32.3	6.7	15.5
± S.E.	±2.9	±1.0	±2.6	±1.1								
C + L	153.8	27.4	139.6	120.0	165.8	214.5	25.7	7.3	22.1	31.0	6.4	15.9
± S.E.	±2.4	±8.1	±2.7	±1.7								
N	159.2	16.1	144.4	122.3	193.8	566.0	69.2	8.7	11.1	79.0	8.9	9.5
± S.E.	±2.6	±0.8	±1.2	±1.5								
N + L	144.4	23.5	142.1	123.2	180.7	619.3	76.3	15.9	17.2	88.0	21.7	19.0
± S.E.	±3.5	±3.1	±1.8	±1.2								

*Calculations:

From muscle per 100 Gm. noncollagenous dry fat-free solids (NCDFFS):

$$(ICW)_{ml.\ skin} = \frac{(K)_{Total} - \left[[K]_{ECW} \times (ECW)_{skin}\right]}{[K]_{ICW\ (muscle)}}$$

$$(ECW)_{ml.\ skin} = (H_2O)_{Total\ skin} - (ICW)_{skin}$$

$$Na_{EX} = (Na)_{Total\ skin} - \left(\left[[Na]_{ECW} \times (ECW)\right] + \left[[Na]_{ICW} \times (ICW)_{skin}\right]\right)$$

$$Cl_{EX} = (Cl)_{Total\ skin} - \left([Cl]_{ECW} \times (ECW)_{skin}\right).$$

reduced increment of noncollagenous nitrogen content of the nephrotic animals, indicating reduction in tissue mass; however, the K/NCN ratio per 100 grams of residual dry fat-free tissue (muscle and carcass) of the nephrotic animals was equivalent to or greater than that of the controls.[98] Therefore, surviving cells had an approximately normal composition.

Based upon the measured intake and observed change in total body composition, a more accurate estimate of urinary excretion patterns during diuresis could be made than was available from the usual rat balance technique. It will be noted that in the nephrotic animals the urinary excretions of sodium, chloride, and potassium during diuresis were greater than in pair-fed controls. The excretion of Cl was considerably greater than that of Na, reflecting the usual chloride excess in an "acid-ash" diet. The nephrotic rats were not completely edema free after diuresis, judging from body contents of H_2O, Na, and Cl.

In another experiment, the effect of Na restriction, with or without dietary depletion of K, was explored. It was reasoned that if cellular defect of Na accumulation and K loss characterizes the nephrotic syndrome, cells would not conserve K and intracellular concentrations of this ion would fall, despite the maximal stimulus occasioned by lack of K intake. The results are shown in Table XI. Group 1 had the usual synthetic control diet; Group 2 a low-sodium diet in which excessive potassium intake was avoided; and Group 3 received a similar low-sodium diet, but completely devoid of potassium. Food consumption was poor as a result of the pair-feeding procedure so that control rats more properly might be considered "inanition controls." Potassium content per unit of protoplasmic nitrogen, $\frac{K}{NCN}$, was slightly increased, particularly in the electrolyte-depleted groups. With reference to the previous data it would appear that the nephrotic syndrome in growing rats is associated with failure to gain or with loss of quanta of protoplasm. While nephrotic rats could not adequately defend cell K concentration in the absence of K intake, nevertheless, the electrolyte content of surviving cells in the nephrotic rat appeared to be considerably better than that of controls. The better defense of K content may have resulted from the endogenous utilization of the potassium and nitrogen derived from either the diminished anabolic or increased catabolic processes in the nephrotic animal, as shown by the reduced amount of total nitrogen and potassium with preservation of normal $\frac{K}{NCN}$ ratios in the surviving tissues of nephrotic rats on potassium-free diets. Analyses of muscle (Table XII) indicated that intracellular K content and intracellular water K concentration in the K-deficient nephrotic rats was significantly greater than in their diet controls. Noncollagenous nitrogen content in the nephrotic group was slightly reduced, and the $\frac{K}{NCN}$ ratio was increased. Note that despite greater Na content in the edematous muscle, intracellular Na concentration was lower in the nephrotic rats which had better intracellular K concentrations. Presumably, lack of equivalent catabolic cell destruction in nonnephrotic controls limited the endogenous source of K and therefore greater deficits occurred in surviving cells of the inanition control animals.

In addition to the previously reported data on muscle composition, recent observations in our laboratories indicate that the edematous nephrotic rat has an extraordinary deficiency of organic phosphate and probably some depletion of magnesium in muscle (Table XIII). The reduced concentrations of organic phosphate and magnesium may be related to the reduced intracellular potassium concentrations with increased intracellular sodium content and concentration previously reported.[92] These data suggest that some metabolic pathway, in which these ions are essential activators or cofactors, might be influenced by reduced concentrations of these ions; or, vice versa, alteration in some metabolic pathway might account for disturbed metabolism of these

ions. Preliminary studies with isolated rat diaphragm support either hypothesis.[100]

DISCUSSION

From the foregoing observations a tentative scheme of the pathogenesis of nephrotic edema may be put forward (Fig. 19). The etiology of the nephrotic syndrome in children in most instances remains unknown. It may result from some immunologic response or alteration of intermediary metabolism or both.[101] The primary lesion apparently occurs in the glomerular basement membrane, which leads to two major series of phenomena: (1) those relating to the kidney per se, and (2) those associated with generalized systemic or cellular changes.

The renal lesion initially produces hemodynamic and permeability changes[2] leading to functional alterations in glomerular filtration rate and renal blood flow[1, 3, 27] which modify both the quantity filtered and the net tubular cell transport of water and solutes. The interstitial edema surrounding postglomerular capillaries or thickening of the capillary wall leads to external and/or internal vascular narrowing. This reduces blood flow and produces relative ischemia of nephrons. Usually this is reversible, and remission may follow. If the phenomenon persists for a sufficient period, structural capillary occlusion may develop and lead to obliteration of nephrons and ultimate death from renal insufficiency.

Within the tubular cell, metabolic energy sequences may be altered, as manifested by disturbed cellular enzyme activities,[102, 103] increased sodium reabsorption, and excessive secretion of potassium and hydrogen ions.[59]

The systemic effects probably are initiated by the increased permeability of the glomerular basement membrane to protein[2, 43] resulting in loss of proteins from the plasma, particularly those of lower molecular weight such as albumin.[73, 104, 105] This loss of protein, in some as yet unknown manner, results in alterations of protein metabolism.[21] On the one hand, increased degradation of albumin

Table X. Changing body composition with accumulating edema and subsequent diuresis

	No. rats	*Weight (Gm.)*	*DFFS (Gm.)*	*H_2O (Gm.)*	*Na (mM.)*	*K (mM.)*	*Cl (mM.)*	*NCN (Gm.)*
			1. Pair-fed controls					
A. Initial	3	46.3	8.64	33.2	2.3	2.9	1.6	0.69
B. 12 days	3	78.8	14.70	48.1	2.9	4.6	2.2	1.35
B - A		+32.5	+6.06	+14.9	+0.6	+1.7	+0.6	+0.66
C. 18 days (Na-free + Diamox)	3	83.4	18.57	55.6	3.3	5.7	2.4	1.68
C - B		+4.6	+3.87	+7.5	+0.4	+1.1	+0.2	+0.33
Estimated urine excretion (6 days)					1.0	9.1	3.9	
			2. Nephrotic					
A. Initial	3	46.3	8.64	33.2	2.3	2.9	1.6	0.69
B. 12 days (aminonucl.)	3	107.5	13.73	70.9	7.1	4.3	5.6	1.16
B - A		+61.2	+5.09	+37.7	+4.8	+1.4	+4.0	+0.47
C. 18 days (Na-free + Diamox)	5	90.6	14.51	60.3	5.2	4.0	4.3	1.28
C - B		-16.9	+0.78	-10.6	-1.9	-0.3	-1.3	+0.12
Estimated diuresis urinary excretion (6 days)					6.7	10.7	8.5	

Table XI. Total body composition*

	No.	DFFS (Gm.)	H_2O (Gm.)	Na (mM.)	K (mM.)	Cl (mM.)	TN (Gm.)	NCN (Gm.)	K/NCN (mM./Gm.)
				Standard diet					
Control	4	11.41	34.66	2.47	3.32	1.61	1.39	1.00	3.32
±	S.E.	0.32	1.24	0.05	0.08	0.06	0.02	0.02	
Nephrotic	4	9.72	31.90	2.40	2.79	1.64	1.15	0.85	3.28
±	S.E.	0.72	1.70	0.14	0.29	0.16	0.09	0.08	
				Low-sodium diet					
Control	9	11.39	34.22	2.58	3.02	1.52	1.34	0.98	3.09
±	S.E.	0.25	1.19	0.09	0.14	0.06	0.05	0.04	
Nephrotic	7	9.58	31.32	2.39	2.82	1.67	1.11	0.79	3.57
±	S.E.	0.36	1.13	0.07	0.11	0.08	0.06	0.04	
				Low-sodium, no-potassium diet					
Control	6	9.17	29.54	2.36	2.42	1.27	1.09	0.78	3.10
±	S.E.	0.43	1.68	0.11	0.12	0.06	0.06	0.04	
Nephrotic	3	7.78	26.34	2.09	2.12	1.48	0.89	0.62	3.42
±	S.E.	1.04	3.37	0.22	0.38	0.17	0.11	0.08	

*Pair feeding of rats in 5 groups: Each group contained nephrotic and control rats on each of the 3 different diets. All rats in each of the 5 groups were pair-fed against the nephrotic rats on the low-Na, no-K diet in their group.

Table XII. Effect of low-sodium, no-potassium diet on muscle composition

	No.	ECW (ml.)	ICW (ml.)	$(Na)_e$ (mM.)	$(Na)_i$ (mM.)	$(K)_e$ (mM.)	$(K)_i$ (mM.)	$[Na]_i$ (mM./L.)	$[K]_i$ (mM./L.)	NCN (Gm.)	K/NCN (mM./Gm.)
Control	7	70.1	278.8	12.3	10.9	0.19	27.4	43.1	98.5	13.22	2.07
±S.E.		2.4	6.3	0.7	0.3	0.05	0.7	2.8	2.5	0.34	
Nephrotic	4	117.1	308.5	18.7	6.5	0.5	33.7	22.9	110.9	12.98	2.60
±S.E.		7.6	34.1	0.8	3.9	0.1	3.7	14.8	10.5	0.50	

Table XIII. Muscle composition in nephrotic rats (per 100 grams DFFS)

	No.	H_2O (Gm.)	P_T (mM.)	P'_i (mM.)	Po (mM.)	Mg (mEq.)
Control	8	347.5 ±18.8	26.6 ±3.2	12.3 ±2.7	14.3 ±3.2	9.2 ±0.7
Nephrotic	8	468.7 ±38.5	27.9 ±3.7	21.4 ±2.1	6.5 ±3.7	8.4 ±1.1

and several other plasma proteins without a significant increase in synthesis has been demonstrated; on the other hand, by contrast, there is a striking increase in synthesis and some delay in degradation of the beta lipoproteins, which have a high molecular weight and are not lost in appreciable quantities in the urine.[22, 106-108] A by-product of altered protein metabolism may be reflected in the altered bone matrix[109] with resultant aberrations in parathyroid gland activity.[110] Increased glomerular permeability to albumin contributes an excessive quantity of albumin to the tubular fluid and may be associated with decreased renal reabsorption of albumin,[111-113] with urinary loss and its associated increase in catabolism. The net effect is a diminution in circulating albumin and a reduction in the total body albumin pool. Plasma colloid osmotic pressure falls[18, 48, 50, 114] and the potential for water distribution across the membrane is changed, leading to an accumulation of water in the extravascular, interstitial fluids.[104, 105, 115] This results in reduction of effective plasma volume.[115] The relative diminution in plasma volume is presumably compensated by elaboration of antidiuretic substances,[80, 116-119]

promoting renal retention of water and resulting in increased body water content with dilution of all solutes contained in the body fluids. Elaboration of sodium-retaining hormone by the adrenals[120, 121] presumably augments the renal retention of sodium which increases solute concentration of the body fluids, and enhances excretion of nonutilized potassium derived from catabolic processes.

In addition to these general systemic effects, the cell substrate may be modified by alteration in the cell protein composition or content and by dilution of the intracellular contents. The cellular concentrations and content of magnesium and of organic phosphate essential for ADP-ATP interactions are reduced. Reduction in essential cytoplasmic enzymes, intermediates, and activating ion concentrations may lead to a net deficit in cell energy yield with reduction in sodium "pump" activity culminating in decreased sodium extrusion[85, 121] leading to an accumulation of sodium in the cell. In some way potassium outflux is enhanced. Despite increased intracellular sodium, a net decrease in intracellular solute may occur with cellular swelling and lowering of intracellular osmolality equivalent to that occurring in the extracellular fluid. Presumably the kidney tubule cell sustains alterations similar to those observed in other tissues, i.e., muscle. The decreased osmolality of the body fluids may be improved as a consequence of the renal retention of additional sodium. The accumulation of this ion in association with water is manifest as edema. The retention of sodium and chloride in the skin may be greater than in the freely exchangeable extracellular fluids. At least one half of the tissue edema may be contained within the skin. The exact nature of the mucopolysaccaride gel in this organ is not completely understood. With the increased volume of body fluids there is a transient improvement in plasma volume although the reduced colloid osmotic pressure is not increased. The persistence of interstitial edema, particularly in the kidney, is partially responsible for the altered intrarenal vascular hemodynamics.

It is clear that the accumulation of edema fluid may be rationalized on the basis both of protein depletion due to increased glomerular permeability and of the excessive retention of sodium and water by the kidneys. It is equally obvious that these phenomena by themselves represent a gross oversimplification of the pathogenesis of edema in the nephrotic syndrome. It is hoped that the hypothetical schema illustrated in Fig. 19 will suggest some areas for further investigation upon edema in the nephrotic syndrome, which may provide a more accurate hypothesis.

SUMMARY

Some physiologic and biochemical observations on children with the nephrotic syndrome and on rats with an experimental nephrotic syndrome are summarized. Particular reference is made to renal function, including some estimates of renal thermodynamics associated with the secretion of potassium by the edematous nephrotic child. Electrolyte balances illustrate the accumulation and diuresis of nephrotic edema fluid with particular reference to the effect of sodium restriction and sodium loading in nephrotic children. The balance data suggest that edema is associated with a decreased intracellular potassium content, as well as an accumulation of Na and Cl extracellularly. Studies of the composition of muscle from a nephrotic child support this thesis. It is suggested that some of the discrepancies in estimating the retention and distribution of Na and Cl by balance measurements may be explained by correction for the retention of sodium, chloride, and water in skin and skeleton. A technique for such correction is illustrated. The skin appears to be the principal tissue reservoir of edema fluid according to such calculations, and some evidence for this is provided by analyses of skin from rats with the experimental nephrotic syndrome. In the edematous skins of these animals, sodium and chloride are retained in excess of their anticipated distribution in the extracellular and intracellular fluids. It is suggested that the "excess" sodium and

chloride might be contained by loose electrostatic apposition to charged polar-end groups in the collagen fibril. Analytical data are given for "excess" Na and Cl of normal human skin, which are consistent with the above concept. Recent studies of cell composition of rats with an experimental nephrotic syndrome indicate a deficit of magnesium and organic phosphate as well as the accumulation of intracellular sodium and decreased concentration of potassium previously described.

An attempt has been made to integrate these observations with others taken from the available literature and other studies by our group upon plasma protein metabolism in the nephrotic syndrome, to construct a tentative, hypothetical schema for the pathogenesis of nephrotic edema.

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Physiologic considerations concerning corticosteroid therapy and complications in the nephrotic syndrome

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THE goals of treatment of the nephrotic syndrome are defined by the physiologic abnormalities in the patient and are a measure of the physician's understanding of the underlying mechanisms involved. The ultimate aim of treatment is complete recovery. The immediate aims, however, are greatly influenced by variations in the clinical expression of the disease and by variability in response to therapy. With the newer therapeutic agents, such as ACTH, corticosteroids, antibiotics, and chlorothiazide, a more optimistic attitude for recovery of the nephrotic child is justified than was held a decade ago. Constant need for hospitalization and severe debilitation of the patient are no longer prominent features of the disease and its management.

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Aided by Grants H-2290 and H-4408, National Institutes of Health, United States Public Health Service.

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Dependence on corticosteroids and antibiotic administration in a routine fashion without knowledge of the pathologic mechanisms effected by therapy and in view of the possible ill effects of therapy per se too often leads to therapeutic failure and unnecessary hardship to the patient. The therapeutic regimen will need to be varied depending upon many factors. For this reason, a review of the basic mechanisms of hypoproteinemia, abnormal lipoprotein metabolism, edema, and disturbed renal function as manifestations of the nephrotic syndrome will precede comments concerning therapy.

HYPOPROTEINEMIA

Increased permeability of the glomerular membrane to serum protein permitting excessive losses of these proteins into the urine appears to be the initial factor in the development of the nephrotic syndrome. Aside from proteinuria, several other mechanisms exist as etiological factors in the hypoproteinemia of these patients.

In the normal child, no more than 100 mg. of protein is excreted in the urine per

day.[1] This protein is, for the most part, globulin. Glycoproteins, a group of proteins containing less than 4 per cent hexosamine and about 1 per cent or 2 per cent of firmly bound carbohydrate, may be found in normal urine in a concentration between 1 and 11 mg. per cent. Other forms of proteinlike material in the urine of normal children which are not coagulated by heat and not precipitated by most of the usual protein precipitants have been identified as mucoproteins[2]; 100 to 150 mg. of these substances are excreted normally each day. The proteinuria in the nephrotic syndrome is composed mainly of albumin (60 to 80 per cent) and an increased quantity of globulin. There may be a small amount of protein not precipitated by trichloracetic acid but precipitated by perchloric acid, e.g., alpha-1 glycoprotein. The glycoprotein content of nephrotic urine has been found to be from 0.0 to 0.2 Gm. per cent in urines containing 2 to 3 Gm. per cent of trichloracetic acid precipitable protein.[3]

Several theories have been advanced to explain the mechanism of proteinuria:

1. Changes in the glomerular membrane allow proteins to pass freely into the glomerular filtrate in abnormally high concentrations and these proteins are eventually excreted into the urine.[4-7] Calculations of the concentration of albumin in the glomerular fluid which were obtained from studies in severely edematous children and adults with albumin and endogenous creatinine clearances[14] suggest that increased glomerular permeability of these proteins is the main cause of the proteinuria. The "calculated" concentration of albumin in the glomerular fluid varied between 100 and 200 mg. per cent when the plasma albumin was raised by albumin administration to values one third to two thirds of normal concentration.

2. A reduction in reabsorption of normally filtered protein by the tubules has been postulated as a cause of proteinuria[1] even when the concentration of protein in the glomerular filtrate is no greater than 25 to 30 mg. per cent.[8-13]

3. It has been proposed that diseased tubular cells may secrete protein into the tubular lumen. To date, this remains a presumptive possibility only.

4. Alteration in the serum protein configuration which could conceivably pass more readily through the glomerular filter has been suggested as a cause for the proteinuria.[1, 12] The question of the similarity between serum and urine proteins in children with the nephrotic syndrome has been answered in part by immunochemical identification of albumin in the serum, urine, and ascitic fluid.[14] Differences between protein fractions in the urine and plasma, however, have been reported by others, demonstrating that the urinary $alpha_2$ and beta globulins contain less lipid, and the $alpha_1$ globulin fraction contains more mucopolysaccharide than their counterparts in the serum.[15, 16] Differences in the protein fractions may exist even when the antigenicity of the molecule, or a portion of it, is identical.

Although increased glomerular membrane permeability particularly to albumin has been shown to exist in the nephrotic syndrome, the question of whether or not a decreased tubular reabsorption of proteins augments the proteinuria is unanswered.[17] Clearance studies of the separate plasma proteins have shown that albumin clearance is the highest, while the $alpha_2$ and beta globulin clearances are the lowest; $alpha_1$ and gamma globulin clearances are between these two parameters. The most common plasma protein alteration in the nephrotic syndrome is a decrease in the albumin concentration. The serum $alpha_1$ globulins are either normal or slightly decreased; the $alpha_2$ and beta globulins are increased while the gamma globulins are markedly decreased. From a study of 6 children with the nephrotic syndrome, with the use of radioiodinated human plasma albumin, human gamma globulin, and iron-binding globulin, the data seem to indicate that the lowered serum protein values are due to an increased fractional rate of catabolism of albumin, gamma globulin, and iron-binding globulin in association with protein losses via the urinary tract.[18] In the patient with moderate hypo-

albuminemia and without ascites but with minimal edema, the hypoalbuminemia was found to be caused by massive renal losses or increased catabolism. In the patients with severe hypoalbuminemia, ascites, and anasarca, the causes of the lowered serum protein concentrations were believed to be increased catabolism and severe renal losses. The rates of synthesis of albumin and gamma globulin were said to be within normal range, or even increased. Carbon 14,[19, 20] sulfur 35, methionine,[21] nitrogen 15, and glycine[22] have been shown to be incorporated at an increased rate into plasma proteins in the nephrotic syndrome, suggesting increased protein synthesis. It has been argued, however, that this increase in specific activity need not necessarily indicate an increased rate of synthesis of proteins, since the turnover rates of plasma proteins in the nephrotic syndrome may be increased.

Nutritional status of the patient with the nephrotic syndrome is often not obvious, particularly with large accumulations of edema and anasarca obscuring the poor tone and small size of the muscles and fat. However, after loss of edema, malnutrition is evident. Although patients have been shown to have positive nitrogen balances, this is difficult to correlate with the evident muscle wasting seen in these children. From the published data, it is not clear at what stage in the disease these nitrogen balance data have been collected. The total noncollagenous nitrogen content of the body of nephrotic rats was observed to be reduced when compared to that of pair-fed control rats.[23] Several investigators[24, 25] have demonstrated nitrogen repletion in nephrotic individuals given high protein intakes. For example, it has been shown that by increasing the protein intake progressively to levels up to 0.8 Gm. nitrogen per kilogram per day, there is a progressive increase in nitrogen retention. It is also interesting to note that urea excretion rises with high protein intakes in the normal person, while in the patient with the nephrotic syndrome, under similar circumstances, the urea outputs were low,[26] further suggesting an increased capacity for nitrogen retention in the nephrotic patient.

The intravenous administration of concentrated human serum and plasma[27-30] to patients with hypoproteinemia with the nephrotic syndrome has been used therapeutically for over two decades. A 25 to 30 per cent increase in the plasma volume, a 10 to 20 per cent rise in serum colloid osmotic pressure, small variable changes in total serum protein concentration, and increased proteinuria were demonstrated following a single injection of 25 Gm. of concentrated serum albumin into 3 patients with the nephrotic syndrome.[31] A large portion of the administered concentrated human albumin could be recovered in the urine; however, when proteinuria diminished as the disease subsided, the injected albumin could be accounted for in the circulation in 3 other nephrotic children.[32] Diuresis was induced in 47 per cent of instances when salt-poor albumin was given in the nephrotic syndrome.[33] Similar results have been observed with plasma substitutes, dextran, or polyvinylpyrrolidone.[34] In one study, the administration of concentrated human serum albumin was associated with a slight rise in serum protein concentration and an associated increase in glomerular filtration rate.[35, 36] This was followed by increased sodium excretion if loss of edema fluid occurred. From this study it was suggested that excretion of a salt-retaining steroid in the urine of patients in the edematous phase of nephrosis decreased after the administration of albumin. Although a question has been raised of possible nephrotoxic effects with long-term albumin therapy, children treated with albumin on a long-term basis were found not to have renal lesions which could be ascribed to albumin therapy.[37, 38] Specific renal functional studies, however, were not reported. One of these was a 3-year-old child who received almost daily infusions of concentrated albumin (10 to 15 Gm.) for 18 months. With these infusions, the child's appetite improved and he was more comfortable. Whenever the infusion was omitted for a day or two, the urine volume fell,

edema increased, and the nonprotein nitrogen promptly rose; with resumption of therapy, there was a return to the previous status. At no time were there untoward reactions except when albumin was injected too rapidly and then an erythematous rash and vomiting occurred. Hypertension during albumin infusion has been noted. This disappears when the albumin administration is discontinued.

In summary, the major cause for the development of hypoproteinemia in the nephrotic syndrome appears to be the renal loss of plasma proteins; increased catabolism of albumin, gamma globulin, and iron-binding globulin may be added factors. Poor dietary intake may be a contributing third factor promoting hypoproteinemia. Adequate protein intake should be encouraged in these patients as it has been demonstrated that by increasing the protein intake up to 5 Gm. per kilogram per day there is a progressive increase in nitrogen retention. The nutritional status of these patients is variable, depending upon length and severity of the active disease. Intravenous administration of albumin has been of value in raising the level of serum albumin and in promoting diuresis in some patients.

LIPID METABOLISM

The serum of patients with the nephrotic syndrome is usually lipemic. This rise in serum lipids usually involves all fractions[39]; cholesterol and neutral fats, however, are increased to a greater extent than phospholipids.[40, 41] There is also an associated endogenous chylomicronemia.[42]

The mechanisms which have been suggested as possible causes for the development of hyperlipemia in the nephrotic syndrome are: (1) increased lipid intake, (2) increased cholesterol synthesis, (3) increased mobilization from fat depots, (4) failure of a "lipid clearing factor," and (5) faulty utilization or degradation of lipids.

The question of increased intake as the cause of hyperlipemia in nephrosis has been adequately answered in the negative, both in the human disease and in the experimental animal. Hyperlipemia and hypercholesterolemia in the nephrotic rat are not of exogenous origin,[43] and other studies[44, 45] have failed to demonstrate increased intestinal absorption of cholesterol in rats with the nephrotic syndrome.

The possibility of increased cholesterol synthesis in the nephrotic syndrome was suggested in a study with the use of deuterium,[46] where the half-life time of the labeled cholesterol was prolonged about 5 times above normal. However, it has been pointed out[47] that the data obtained with the use of deuterium, during a steady state, could be explained either by increased synthesis of cholesterol or a decrease in the rate of removal, or both. From studies of the rate of hepatic synthesis of cholesterol by measuring the rate of biliary excretion of cholesterol, the nephrotic hypercholesterolemia could not be ascribed to an increase in the rate of cholesterol synthesis by the liver.[48] Moreover, the disappearance slopes of C^{14}-labeled cholesterol in 2 nephrotic patients was observed to be the same as in the normal control.[49] Comparable values were noted by the use of C^{14}-labeled acetate in extracts of liver slices obtained from control and nephrotic rats which were killed at 8, 24, and 48 hours after the injection of nephrotoxic serum.[50] From these studies, it was concluded that there was no evidence to support the hypothesis of an increased rate of cholesterol synthesis in the nephrotic rat.

The question of increased mobilization from the fat depots in the body has been studied[51] and evidence is presented to show that in the severe nephrotic syndrome experimentally induced in rats there is a redistribution of lipid within the body and an associated mobilization of lipemia, while in the milder forms of the disease the hyperlipemia is the result of increased hepatic lipogenesis.[52] Other investigators,[53] however, found no accumulation of lipid in any part of the body except for the plasma, and also showed that the content of lipid and of cholesterol in the liver of nephrotic rats was similar to the content in the normal ones. These latter authors, in order to study the

effect of starvation per se on hyperlipemia, administered nephrotoxic serum to starved rats which were depleted of depot fat and found an even greater degree of lipemia and of hypercholesterolemia than that observed in the nonstarved nephrotic animal. Since the accumulation of lipids in the nephrotic rat is first demonstrated in the plasma compartment,[54] it was felt that perhaps the loss of albumin initiated the hypercholesterolemia and hyperlipemia. It was further observed[55] that the rise of plasma lipids did not occur unless there was an external loss of some plasma constituent in the urine. This was demonstrated by preventing urinary loss either by ureteral ligation or ureter–vena caval anastomosis. Giving albumin to the nephrotic rat inhibited or completely prevented the hyperlipemia. Thus, it was suggested that the hyperlipemia and hypercholesterolemia occurring in experimentally induced nephrosis resulted from the deficiency of circulating plasma albumin. Another contributing factor may be the renal loss of heparin or its plasma equivalent, lipoprotein lipase, a lipid clearing factor.[56] Recently, it has been shown[57] that hyperlipemia and hypercholesterolemia may precede hypoalbuminemia in the nephrotic rat and these investigators felt that a causal role of hypoalbuminemia in nephrotic hyperlipemia does not exist.

A final consideration involves faulty utilization or degradation of lipids. The lipoproteins of the sera of normal adults and of nephrotic patients[41, 58-60] have been separated into several fractions by ultracentrifugation at different densities. The nephrotic patient has been categorized into three groups by the variations in the densities of the lipoprotein fractions as shown in Table I.

In general, the patients in Group 1 have been associated with the lowest level of serum albumin and the more severe forms of the disease. Group 3, on the other hand, are patients with higher serum albumin levels and less marked edema and ascites. These studies also showed that the lipoprotein composition of serum could be predicted from lipid analyses of whole serum. If the ratio of the total cholesterol to total triglycerides of the whole serum is less than one, then the predominant elevation is in the very low density fraction ($D < 1.019$). Since increase in the very low density fraction with a high content of triglycerides is responsible for the lactescence of serum, the lipoprotein composition of serum can be predicated with reasonable accuracy from the appearance of the serum and the cholesterol level. Elevation of the lower density beta lipoproteins, primarily the SF 15 to 400 class, have been found to be associated with hyperlipemia and hypercholesterolemia.[60] Under these circumstances the concentration of the alpha lipoproteins and higher density beta lipoproteins SF 3 to 8 are either normal or decreased. With the use of I^{131} the half life of alpha lipoproteins was found to be accelerated. The fractional rate of conversion of the lower density beta lipoprotein to higher density which occurs in the normal child was decreased in the nephrotic child, giving rise, in part, to the hyperlipemia observed in nephrosis. Catabolism (half-life) of the higher density beta lipoproteins, which in the normal child is about 3 days, was a bit accelerated in the nephrotic child, while the conversion and catabolism of the low density beta lipoprotein were similar to those observed in normal individuals.

It would appear from observations during remissions and exacerbations of the nephrotic syndrome that in general the serum lipids and serum albumin levels show an inverse relationship. When proteinuria clears, the elevated serum lipids and cholesterol levels return to normal. However, patients have been observed in whom the elevated serum cholesterol levels remained for some time after the proteinuria has cleared. An inverse relationship was found between the serum cholesterol and albumin levels following albumin infusions over a period of 4 weeks in nephrotic patients.[37] This relationship seemed to hold also for the total lipids as demonstrated in two such studies, one in a 14-year-old girl with clear serum and in a 36-year-old man with milky serum.[37] The basis for this effect of albumin on the lipo-

Table I

	Density of lipoprotein fraction		
	< 1.019	*1.019-1.063*	*1.063-1.210*
Group 1	++++	0	0
Group 2	++	++	0
Group 3	+ to 0	+++	+ to 0

proteins in the nephrotic syndrome remains obscure.

EDEMA

It is generally agreed that edema in the nephrotic syndrome follows in time relationship to the proteinuria, which is a consequence of the glomerular lesion. Albumin is the main protein lost in the urine (often amounting to 5 Gm. or more per day). This urinary loss, plus the daily replacement needs for albumin which are approximately 8 Gm. per square meter per day,[61] based on a value of 13 Gm. per day for the adult with an albumin pool estimated at 500 Gm. and with the increased catabolism of albumin of the nephrotic syndrome, leads to a negative albumin balance producing hypoalbuminemia. The resultant decrease in plasma albumin leads to a decrease in colloid osmotic pressure which is a factor, according to the Starling hypothesis, leading to edema formation. A tendency toward hypovolemia develops which is considered by some investigators[62] to be the stimulus for water and sodium retention. A more detailed discussion concerning other possible and additive factors which increase the tubular reabsorption of water and electrolytes, such as glomerulotubular imbalance,[63-65] increased intrarenal pressure,[66-69] increased capillary permeability,[70] the effect of antidiuretic hormone,[71] aldosterone,[72] and autonomic nerve stimulation[73] is presented in another section of this symposium. These factors are quite important in explaining the occurrence of diuresis in those patients who have spontaneous diuresis without showing significant changes in plasma protein concentrations.

While it is true that corticosteroid therapy is a most effective agent in relieving edema of the nephrotic syndrome, a discussion of other agents which promote diuresis is of importance, particularly under the following circumstances: (1) failure to respond to steroid therapy, (2) where steroids are contraindicated, and (3) to effect temporary improvement prior to the use of steroid treatment. A consideration in the treatment of edema is the symptomatic manifestation of the fluid accumulation and the areas in which the edema fluid may be loculated. If respiratory embarrassment is present as a result of ascites or hydrothorax, then a paracentesis or a thoracentesis is indicated.

Salt restriction in the nephrotic patient with edema has been in use for over five decades.[74] The renal sodium excretory capacity of the nephrotic patient is variable and is related to edema formation. During edema accumulation, a low salt diet is indicated.[62, 75] With diuresis, sodium is eliminated adequately and no salt restriction is necessary; indeed, sodium administration may be necessary if hyponatremia persists after large losses of edema fluid have occurred. Only 3 patients of 30 treated with a low sodium–high potassium diet (2 to 9 mEq. of sodium, 150 mEq. of potassium, and 3 Gm. of protein per kilogram of body weight each day) with and without corticotropin, nitrogen mustard, and plasma expanders[70] developed serum sodium concentrations below 132 mEq. per liter. There is no clear indication for reducing the sodium intake in edematous patients who have reached a steady state and in whom the plasma volume has become stabilized. On the other hand,[76] the administration of sodium and potassium salts promoted diuresis which occurred when the plasma sodium concentration was raised to 140 mEq. per liter and the urine sodium concentration to 200 mEq. per liter. It becomes apparent that during the "steady state" in the edematous patient with the nephrotic syndrome, there is little indication to reduce drastically the sodium content in the normal diet. Cation exchange resins[77, 78] have been used to decrease intestinal absorption of sodium and, in some patients, to induce diuresis. However, electrolyte and

gastrointestinal disturbances may result.[79] Hyponatremia due to extracellular dilution, intracellular sodium transfer, and poor dietary intake may need correction with sodium and potassium administration to restore body composition and osmolarity.[80] Water restriction or high fluid intakes have no value in the treatment of the nephrotic patient and may even be harmful.

Attempts at promoting sodium excretion with mercurial diuretics may be effective[81] but they are not widely used in the pediatric patient with the nephrotic syndrome. The administration of ammonium chloride or urea to decrease renal tubular reabsorption of sodium enjoyed popularity prior to the steroid therapy era.[82] Chlorothiazide, a benzothiodiazine derivative, is an organic sulfur which has the ability to inhibit reabsorption of sodium and chloride by the renal tubule and thus induce diuresis.[83, 84] Doses of 20 mg. per kilogram per day have been reported to produce adequate chloruresis and natruresis in most patients. When the patients are given adrenal steroid therapy in addition, potassium excretion predominates. Reduced glomerular filtration rates and low serum albumin concentrations (below 1.5 Gm. per cent) may make for drug refractoriness. Higher doses of chlorothiazide apparently do not increase its effectiveness. Marked potassium excretion has been reported[85] with this drug, with resulting low serum potassium. The administration of chlorothiazide can be a valuable adjunct in the treatment of the edema of the nephrotic syndrome.

Elimination of edema fluid has been effectively accomplished with the use of plasma expanders, such as dextran or polyvinylpyrrolidone, and by the repeated administration of albumin.[34] Dextran (12 per cent solution in distilled water) given intravenously at a dose of 300 to 400 ml. per square meter daily, or on alternate days at a rate of 2 to 4 ml. per minute, produces a water diuresis which is preceded by an increased sodium excretion following an increase in the plasma volume. The diuresis is sensitive to Pitressin. Contraindications to dextran therapy are preexisting hypertension and massive anasarca. If during the infusion the blood pressure rises above 140 mm. Hg, the dextran should be discontinued. Maximum benefits are achieved when 3 to 5 consecutive infusions are used.[86] Polyvinylpyrrolidone (PVP), 500 ml. of a 3.5 per cent solution given daily during a 4 to 6 hour period, induces diuresis in most nephrotic children. The effect of administered concentrated human albumin has been discussed earlier in this report. It may be worth noting again that, unless albuminuria is suppressed, the effects of albumin administration will be of transient value. When concentrated albumin solutions are given for a short period of time (less than a week) a considerable portion of the administered dosage is retained; however, when given for longer periods and as a result of greater spillage in the urine, the administration is less efficient.[87]

Fig. 1 represents the data obtained from a 4-year-old child who, inadvertantly, was given large doses of triamcinolone (96 mg. per square meter per day) over a period of 3½ months. He became excessively edematous. With administration of 25 Gm. of albumin a day for 7 days, the child showed no response; the serum albumin remained at 0.2 Gm. per cent. However, by increasing the albumin dosage to 75 Gm. per day for 14 days, this child lost most of his edema (approximately 35 per cent of his body weight) and the serum albumin concentration rose to 2.2 Gm. per cent. His urinary albumin excretion at this time was about 12 Gm. per day. The following week when albumin was not given, the urinary albumin excretion decreased to 3 Gm. per day.

The use of steroidal antagonists of aldosterone in counteracting edema formation has been recently reported.[88, 89] These reports indicate that certain synthetic steroids act by increasing the urinary sodium excretion. In the absence of sodium-retaining steroids these aldosterone antagonists have little effect. In the presence of a sodium-retaining steroid, however, a marked sodium diuresis is observed and, interestingly enough, there is no increase in potassium excretion in

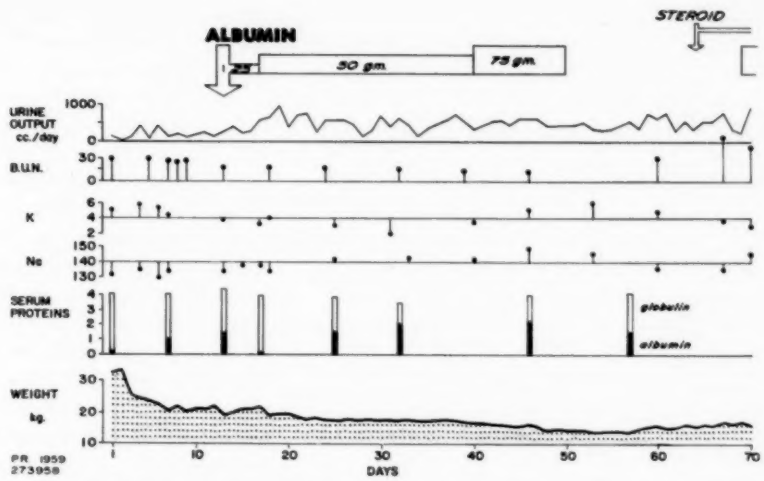

Fig. 1. P. R. is a 5-year-old boy with the nephrotic syndrome since the age of 16 months. After partial response to prednisone (persistent proteinuria), he was placed on maintenance steroid therapy at 18 months of age for the ensuing 2½ years, during which time he continued to show some albuminuria and mild edema. Three and a half months prior to admission, he was placed on continuous triamcinolone therapy ranging from 64 to 96 mg. per square meter per day (an excessive amount of steroids compared to the usual steroid dosage schedule used in our clinic). He began to accumulate edema fluid very rapidly and was finally admitted to the Buffalo Children's Hospital with extreme anasarca and marked hypoalbuminemia and signs of heart failure. On admission, steroids were withdrawn and abdominal paracenteses were performed to relieve the intraabdominal pressure. He was then placed on a regimen of 25 Gm. of salt-free albumin per day without any obvious improvement. Increasing the daily administration of albumin to 50 Gm. per day and later to 75 Gm. per day resulted in diuresis with a gradual weight loss, rise in the serum albumin levels, and a decrease in the blood urea nitrogen. The hypokaliemia which developed while he was receiving 50 Gm. of albumin per day may have been associated with chlorthiazide which he received during that period. Following preparation of this patient in this manner, it was considered safe and appropriate to reinstitute steroid therapy at this point.

the urine. Results of a study[90] including 4 children free of renal and adrenal disease and one 12-year-old child with chronic hypokalemic alkalosis without hyperaldosteronism suggested that the mechanism of action of the sodium diuresis which occurs with the aldosterone-antagonist is not directly related to aldosterone per se, but to an altered renal tubular hydrogen ion excretion. In 4 nephrotic children, diuresis was not effectively produced with Spirolactone, the aldosterone antagonist, at an oral dose of 25 mg. per kilogram body weight.[91]

RENAL FUNCTION

Measurements of discrete renal functions in the nephrotic syndrome show great variability. Renal clearances measuring glomerular filtration, renal blood flow, and tubular excretory function and their ratios to each other may be normal, low, or elevated.[92-96] The clinical symptomatology of azotemia and acidosis cannot be directly correlated with these functional tests of the kidney. Such signs of nephritis as hematuria, azotemia, and hypertension may be associated with the nephrotic syndrome.[97-100] Early in the course of the nephrotic syndrome in children, marked disturbance of the functioning nephron is not common.[101, 102] When persistent azotemia, hematuria, and hypertension are associated with a persistently low rate of glomerular filtration, the prognosis is poor.[102] Long-term studies[103] have dis-

closed various functional patterns in the clinical course of the nephrotic syndrome. Three categories of patients were described: (1) progressive glomerular and, eventually, tubular insufficiency terminating in death within one to two years, (2) latency with periods of exacerbations which in a high percentage of patients resulted in renal insufficiency, and (3) complete recovery without permanent impairment of renal function. It was apparent, in some, however, that classification by means of renal functional studies could not be made with assurance until the full course of the disease was complete. Nineteen of 21 patients studied without evidence of glomerulonephritis* (Group 3) had normal or above normal glomerular filtration rates. However, 2 of these with normal filtration rates later showed a depression in the glomerular filtration rates with the following complications: thrombosis of the inferior vena cava and left renal vein and massive edema (with loss of edema, the filtration rate measured 3 months later rose to above normal levels). Intercurrent infections were often associated with lowered functional status. Persistent functional insufficiency may not become manifest until later in the course of the nephrotic syndrome.

Prior to the development of advanced failure, attention to certain discrete renal functions may be helpful in management. The basal water intake requirement in renal insufficiency is generally determined by and equal to the insensible water losses (500 to 1,000 ml. per square meter each day) plus the urinary output. However, more exacting measures have been used, such as the estimated obligatory water requirement at the very low concentrating capacities seen in renal disease, which is about 85 ml. per 100 calories metabolized.[104] An increase in the solute load excreted will place a demand on body water stores if sufficient water is not supplied. Thus, milk formulas with a low protein content which present smaller quantities of solutes for renal excretion are less demanding of obligatory renal water. When non–protein-containing parenteral solutions are used as the only supply of food, the renal obligatory water needs are reduced to about 35 ml. per 100 calories metabolized, since the solute load demanding excretion is low.

Sodium requirements are variable, depending upon the excretion patterns. In general, when patients are accumulating edema, urinary sodium excretion is reduced and sodium intake must be restricted. When there is a stabilization of edema, sodium excretion may parallel the sodium intake.[105] During diuresis, large amounts of sodium may be lost and body stores may become depleted if extra sodium is not supplied.

The potassium intake requirements in chronic renal insufficiency vary with the body stores, renal excretory patterns, plasma levels, and other indices of altered potassium metabolism, such as the electrocardiogram. Under normal circumstances, maximal rates of excretion for potassium exist when body stores are satisfied,[106] and potassium is conserved when the stores are depleted of potassium.[107] However, in the presence of renal tubular dysfunction and where there is wastage of base as a result of a decrease in hydrogen ion exchange and ammonium ion production, potassium excretion may continue at high rates in the presence of depleted potassium stores. Sodium administration to normal subjects maintained on low salt diets promotes potassium excretion. With significant amounts of the administered sodium entering the cell and displacing potassium, the sodium intake influences the potassium stores and requirement.[108] With an adequate urine volume, potassium intoxication is usually not a problem. It should also be noted that severe potassium depletion in itself causes renal damage with loss of concentrating capacity (distal portion of the nephron). Even in the presence of chronic renal failure, severe potassium depletion needs correction. This must be done with great care in order not to induce potassium intoxication. Potassium salts are best given orally; 1 ml. of a 10 per cent solution of potassium citrate

*Glomerulonephritis was diagnosed when persistent azotemia, hematuria, or hypertension was present.

contains 1 mEq. of potassium and is well tolerated. It is difficult to determine the body potassium deficits from serum potassium levels alone or from the electrocardiogram, yet in practice these determinations are used as guides in potassium administration. In the presence of hyperkaliemia, rectal administration of 100 ml. of an ammonium carboxylic cation exchange resin,* which can be administered as often as every 4 hours, usually reduces the plasma potassium to safe levels; often a single instillation suffices. Intravenous administration of glucose and insulin, hypertonic sodium chloride, or sodium bicarbonate also may be helpful in reducing the level of plasma potassium. Dialysis and peritoneal irrigation may be used when other simpler techniques have failed.

In patients with acute renal failure, the per cent of filtered phosphorus reabsorbed by the tubules is markedly diminished, and since administration of parathyroid hormone does not increase the phosphorus reabsorption, the defect must reside in the renal tubules.[109] Acidosis is present in uremic patients, but is of no major consequence unless it is severe. When patients are in severe acidosis, sodium and potassium base therapy in excess of chloride, preferably by mouth, is indicated. It should always be remembered that correction of acidosis in the presence of hypocalcemia may lead to tetany, therefore, a calcium salt should be administered whenever acidosis is to be corrected. The blood calcium level is usually low in chronic renal insufficiency, due either to reduction in the protein-bound fraction and/or to a reduction in serum ionized calcium; however, hypocalcemic tetany is uncommon. With an elevation of the plasma phosphorus concentration, the administration of aluminum hydroxide is indicated (5 Gm. will remove about 15 mg. of dietary phosphorus) to decrease intestinal absorption of phosphorus and to aid in the prevention of tetany.

Anemia, a common feature in renal failure, may call for treatment. Bone marrow depression, increased hemolysis of red blood cells, iron deficiency, and capillary leakage may be underlying causative factors. Transfusion of red cells seems to be the most effective way of raising the hemoglobin level, particularly when it is below 8 Gm. per cent. Reactions to transfusions are more common in the presence of renal failure; small transfusions of washed red blood cells may be advisable. However, certain hematopoietic substances (iron, liver, folic acid) also may be indicated.

Hypertension may occur and be intermittent in chronic renal failure in the nephrotic syndrome. It may develop during the administration of plasma expanders, or as an untoward reaction to steroid therapy. Hypotensive agents (Rauwolfia derivatives, Apresoline, magnesium sulfate) are effective except in the late stages of the disease. Ganglion-blocking agents may have some value in severe resistant hypertension. When hypertension is persistent, the long-term prognosis is poor.

The heart usually is not involved intrinsically in nephrosis. Early ascites and pleural effusion may distort the cardiac configuration. Intrinsic cardiac pathology usually accompanies persistent hypertension and uremia. Cardiac failure may follow overzealous transfusion or administration of electrolyte solutions, and may be initiated by potassium intoxication. Digitalis therapy is indicated in congestive heart failure. In the presence of potassium deficit, digitalis intoxication may result from usual therapeutic dosages.

Amino-aciduria and glycosuria have been described during the stage of chronic renal failure in the nephrotic syndrome.[110] In such instances, addition of vitamin D along with the intermittent steroid therapy has reduced the amino-aciduria and improved the hydrogen ion exchange mechanism.

Administration of ACTH and adrenal corticosteroids has been shown to produce marked improvement in renal function during diuresis with an increase of the glomerular filtration rate from ½ to 2½ times the control values.[111] Even in patients

*Smith, Kline & French Laboratories.

with normal glomerular filtration rates, an increase could be obtained with hormone therapy in the nephrotic syndrome. Associated with this rise in glomerular filtration rate is a significant increase in plasma volume and diuresis. Following diuresis, the glomerular filtration rate may decrease and level off above control values. Maximum tubular excretion of p-aminohippurate also increases with diuresis, but the effect is less than that observed on the glomerular filtration rate. It has been shown that histologic improvement of the glomerular lesion occurs in 90 per cent of patients treated with steroids 20 per cent of the entire group (4 of 16) showed a marked healing of the glomerular lesion.[112]

CORTICOSTEROIDS

After a decade of experience with corticosteroid therapy in the nephrotic syndrome, it has become widely accepted that ACTH and the adrenal corticosteroids are agents of first choice in the treatment of the nephrotic syndrome in childhood. Before employing steroids in treatment, the possible complications of such therapy must be understood. A recent extensive review[113] and other publications[34, 114] give detailed discussions of reported untoward reactions with steroid therapy in a variety of pediatric disorders. It is the purpose of this report to include only those reactions in association with steroid therapy in the nephrotic syndrome. These agents produce retention of sodium, chloride, and water and renal loss of potassium. Retention of water and sodium may aggravate existing anasarca and further embarrass respiratory and cardiac function. Increased loss of potassium may result in signs of potassium deficiency and in specific renal tubular damage. Although prednisone and triamcinolone are reported to produce less fluid retention than do ACTH and cortisone, instances have been described of large fluid retention with these agents. Reduction[80] of serum sodium levels below 130 mm. per liter, of potassium levels below 3.0 mm. per liter, and of levels of calcium and chloride below 3.0 mEq. per liter and 90 mm. per liter, respectively, have been reported following steroid therapy. In this same group of patients 32 per cent of the courses of ACTH therapy resulted in hyponatremia ($<$ 130 mEq. per liter). Most of the patients developing hyponatremia were on very low sodium intakes. Oliguria and water retention unrelated to sodium retention are often exaggerated in the first few days of steroid therapy,[115, 116] sometimes leading to water intoxication and convulsions. With diuresis, water is excreted first, followed by sodium excretion and a rise in serum sodium concentration to normal levels. With extensive diuresis peripheral vascular collapse and dehydration may be a consequence. Parenteral fluid administration may be necessary with this complication. Low levels of serum potassium ($<$ 3 mm. per liter) have been reported and are usually observed toward the end of steroid therapy. Where there is a greater tendency toward large urine volumes and increased potassium losses with diuresis, serum potassium may fall to low levels, causing weakness of the muscles, slowing of the heart, and characteristic electrocardiogram changes of hypokalemia; potassium therapy may be indicated under these circumstances. With increasing oliguria during steroid therapy, the serum potassium may rise. Azotemia is common during steroid therapy before the onset of diuresis. The rise in blood urea nitrogen is associated with an early reduction in glomerular filtration rate.[117] This rise in the level of urea is usually not clinically important unless renal failure impends. Hypochloremia is usually associated with hyponatremia. In certain instances, hypochloremia and hypokalemia may coexist. Two instances are described of hypochloremic, hypokalemic alkalosis occurring during rapid diuresis with steroid therapy.[118] One child had severe convulsions and the other demonstrated a Chvostek sign; both became comatose. The first child has become severely mentally retarded, the other child has developed an abnormal electroencephalogram. Neither patient was hypertensive and their spinal fluids were normal. This complication, if due to electrolyte im-

balance, should be preventable. In a study comparing the frequency of acid-base disturbances in nephrotic children treated with steroid therapy and with those not so treated, it was shown that a slightly greater percentage of imbalance occurred in the nonsteroid group.[119] However, the non–steroid-treated group had a higher incidence of azotemia and hypocalcemia.

Central nervous system disorders occurring during steroid therapy have been well documented.[113, 114] Although increased coagulability of the blood has been reported[120, 121] following the use of steroid therapy, no instance of a thrombotic episode was observed in 86 nonnephrotic patients with advanced cardiovascular and/or cerebrovascular disease during the course of hormonal therapy given over a period of 3 weeks or in the posttreatment period.[122] The dose of 200 mg. per day of cortisone used in this study was in excess of the dosage said to be effective in producing thromboembolic episodes (100 mg. per day).[123] Thrombotic episodes may be a manifestation of the nephrotic syndrome, and, from one report, there seems to be no increase in frequency with steroid therapy.[87] Figs. 2, 3, and 4 represent data obtained from a 6-year-old child with the nephrotic syndrome associated with extensive atherosclerosis (calcification of coronary vessels, adrenal, and aorta) and cerebral infarcts. We are unable to ascribe this vascular complication to the nephrotic syndrome per se or to the steroid therapy, since atherosclerosis is not a feature of either.

Hypertension can occur during the administration of adrenal corticosteroid therapy,[113, 114, 119, 124, 125] and lead to encephalopathy with convulsions and permanent brain damage in some cases. Three nonnephrotic children while on prolonged steroid therapy had symptoms typical of a brain tumor, i.e., severe headache, anorexia, nausea, intractable projectile vomiting, diplopia, and neurological signs, pyramidal and extrapyramidal.[125] They also had papilledema, cranial nerve palsies, obesity, and "moon" facies without hypertension. Cerebrospinal fluid pressure was elevated. Doses of steroids were relatively small. The basis for this acute increased intracranial pressure is unknown. A similar episode was observed recently in a child admitted to the Buffalo Children's Hospital with the nephrotic syndrome (C. G., a girl, 2 years and 10 months of age who had had nephrosis since the age of 18 months). Triamcinolone, 20 mg., was used for 9 days and effected a weight loss of 1.7 kilograms and a moderate decrease in albuminuria. She was then maintained on 4 mg. triamcinolone 3 days each week for a period of 2 months without an adequate response. After 8 months of continuous low dosage steroid therapy, she was readmitted because of irritability, vomiting, and strabismus of 3 days' duration. Flushed malar eminences, papilledema bilaterally, and sixth nerve palsy were the only physical findings. Urinalysis, blood serum determinations, and x-rays of the skull were normal. It was felt that a posterior fossa tumor was the likely diagnosis and a ventriculogram was recommended. The ventriculogram revealed a normal ventricular system. Steroids were withdrawn and the patient was discharged on no medication 2 weeks later, the bilateral sixth nerve palsy and papilledema still persisting. Within 2 months after leaving the hospital, the dizziness, papilledema, and strabismus cleared. It was felt that these symptoms and signs simulating cerebral tumor could possibly be explained as a side effect of steroid therapy. Pseudotumor symptoms have been reported to have occurred without steroid therapy.[127]

It may be difficult to determine whether or not convulsive seizures are due to the basic disease or result from steroid therapy. They may occur in the absence of hypertension.[113] Seizures may disappear when steroids are discontinued and with the administration of antihypertensive agents when indicated. Convulsions occur about 3 times as frequently in patients on steroid therapy.[119] Other well-known central nervous system disturbances resulting from steroid therapy include mental and emotional changes ranging from mild emotional instability to psychotic episodes. It may be difficult to indict the hormones since existing latent tendencies may become

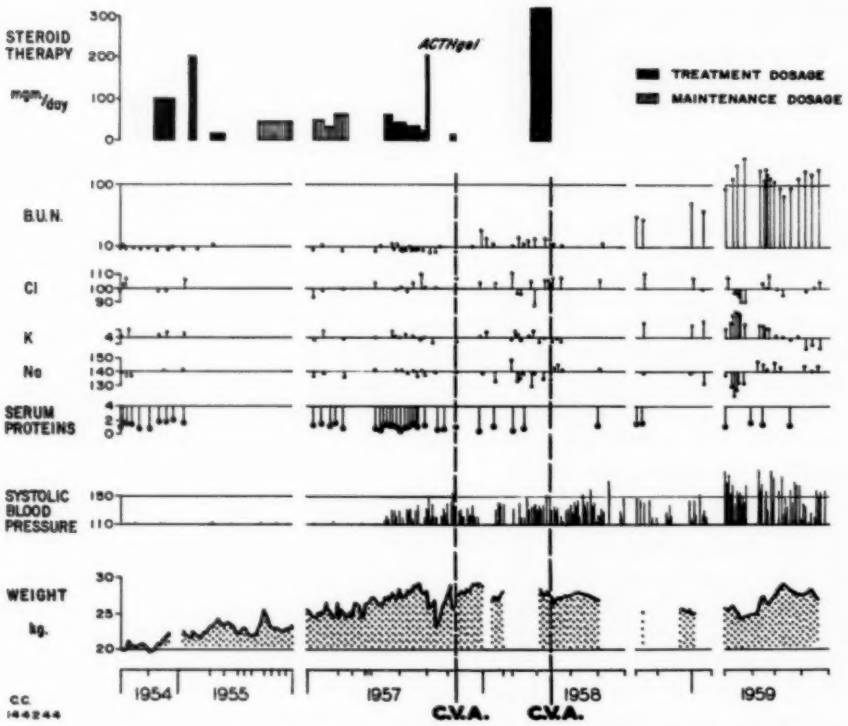

Fig. 2. Shows the development of hypertension approximately 3 years after the onset of the nephrotic syndrome. The hypertension progressed significantly over the next 2 years. It is not clear whether or not hormonal therapy influenced either the hypertension or the vascular changes. The blood pressure rise seems to have begun while the patient received relatively low dosages of cortisone.

manifest with such therapy. Withdrawal of the hormones results in relief of symptoms.

Increased susceptibility to infections has been observed in the nephrotic syndrome without the use of steroid therapy, and death in the nephrotic syndrome due to fulminating cellulitis during the course of steroid therapy has been reported.[128] However, no greater incidence of infection has been shown to occur with the use of steroid therapy.[34, 119] The incidence of serious infection in the nephrotic child has been greatly curtailed with the use of antibiotics. Children with the nephrotic syndrome have been reported to develop a lower antibody response to pneumococci[129] and pneumococcal polysaccharide administration when compared with normal children.[130] Antibody response as measured by hemoagglutination-inhibition tests 2 weeks following a subcutaneous injection of polyvalent influenza-virus vaccine to 44 children with the nephrotic syndrome, 17 of whom were treated with steroid therapy, appeared to be similar in both groups.[131] Children have been observed to develop erysipelas and cellulitis both during steroid therapy and without steroid therapy, with a good response to antibiotic therapy; in some instances, the infection necessitated temporary discontinuance of hormone therapy. Sudden withdrawal of steroid therapy, because of infection, has produced in 2 children with nephrosis either a shocklike state or encephalopathy, which disappeared when steroid therapy was restored.[132, 133] Because of the danger of intercurrent infections, steroid therapy is a calculated risk. Moreover, greater vigilance becomes necessary in

the face of infection since the usual clinical manifestations may be masked. The previously accepted opinion that infections are usually caused by pneumococci, staphylococci, or streptococci is probably no longer the case. In a recent report, gram-negative bacteremia predominated.[134]

The use of steroids commonly produces symptoms like those of Cushing's syndrome, but this is reversible after steroids are discontinued even after long periods of therapy. Gastrointestinal perforation, although uncommon, can occur in the older child.[113, 135] Growth failure frequently seen with continuous steroid therapy[136] is not a constant feature of intermittent therapy, (45 mg. per square meter per day was the minimum dose of cortisone required to reduce growth rate in endocrine-normal nonnephrotic children). Osteoporosis of bones and diabetes mellitus[113] seen with steroid therapy used in other pediatric disorders are rarely recorded in nephrosis.

With early reports showing that diuresis can be induced with ACTH or steroid therapy, the following question remained, "Do these drugs alter the natural history of the disease?" This question has been answered in part by showing that these drugs not only promote diuresis but alter the glomerular basement membrane lesion.[112, 137] Such evidence presented demonstrates that those patients responding to steroid therapy with diuresis and reversal of chemical values to normal also show morphologic responses of the glomerular membrane lesion. A regimen for treatment of a child with the nephrotic syndrome must be individualized depending upon the magnitude and severity of the disease and the abnormality of physiologic expression. Certain basic principles for therapy are generally accepted:

1. Steroid therapy should be started as soon as the diagnosis of idiopathic nephrotic syndrome is made.

2. Initial treatment consisting of either ACTH or steroid therapy should be continued long enough to effect a diuresis, a return toward normal biochemical status, and restoration of renal function.

3. Following biochemical remission, intermittent steroid therapy is given 3 to 4 days of each week for 6 to 12 months.

There is still minor controversy as to whether children with the nephrotic syndrome should receive steroid therapy. When edema, hypoalbuminemia, proteinuria, and hyperlipemia exist, there is little question generally that they should be given steroid

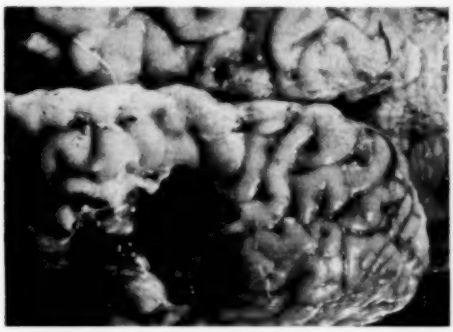

Fig. 3. Patient C. C. Gross appearance of exposed brain demonstrating cerebral infarct in the left occipital region, measuring 3 cm. in diameter. Areas of calcification were noted throughout the brain.

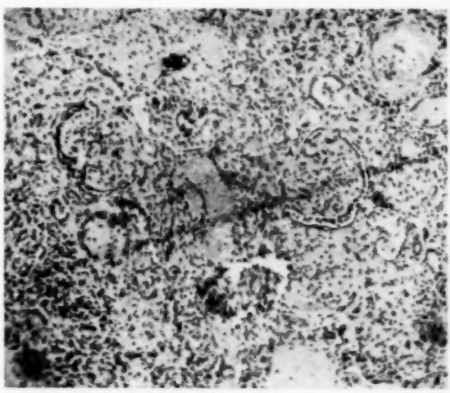

Fig. 4. Patient C. C. Microscopic section of kidney. Note the extensive hyalinization of the glomeruli. No normal glomeruli were found in the kidneys sectioned. Capsular thickening is evident, and the stroma shows diffuse lymphocytic infiltration in the interstitial tissue of medulla and cortex. The arterioles are thickened and, in part, hyalinized. (Original magnification ×120).

therapy. Several instances have been recorded of patients with nephrosis in whom edema is a late manifestation (several years) or never develops. Most edema-free patients do eventually develop edema. It is also important to determine if an underlying disease process is present such as lupus erythematosus, diabetes mellitus, renal amyloidosis, renal vein thrombosis, or Henoch-Schonlein purpura which may not respond as effectively to therapy. If tuberculosis is present, antituberculosis therapy is necessary, particularly with the use of steroids. If severe renal failure exists or if there is great distortion of plasma chemical values, then steroid therapy should be avoided until some improvement in renal function or plasma chemical values can be established by the use of other methods as discussed above. In moderate degrees of renal insufficiency, a good response to steroid therapy is not precluded.[114, 138]

There is no clear evidence of a beneficial effect of corticotropin over adrenal corticoid therapy. Since the steroids can be administered by the oral route, there is a decided advantage in using this form of therapy. The dosage schedules generally used have been calculated either on a surface area basis, estimated dry body weight, or by some other empirical means. On occasion, poor absorption can account for a failure in response, but this can be overcome by increasing the dosage of steroids. Many reports have documented the results with ACTH[80, 130-147] and with steroids[124, 135, 148-153] for initial treatment. By calculating on an estimated dry weight basis, we have used cortisone 300 mg. per square meter per day, prednisone 60 mg. per square meter per day, and triamcinolone 30 mg. per square meter per day as initial treatment doses. The total calculated daily amount of steroid is given in 4 divided doses throughout the day. Usually within 3 weeks, the proteinuria and edema have cleared and the blood chemical values except for the cholesterol have returned to normal. However, if diuresis has not been established by the third week one might elect to continue therapy for a longer period, increase the dosage of steroids, or stop the therapy for a short period and then give another full course of therapy. In some instances, corticotropin therapy, 1 mg. per pound of body weight per day, has been effective when a course of steroid therapy has failed. Recently, success has been reported with the combined use of ACTH and mechlorethamine (0.1 mg. per kilogram) in the face of failure with steroids or ACTH alone.[154] Combinations of steroid therapy and intravenously administered albumin may prove effective in resistant cases. The artificial induction of malaria[155] and intravenous T.A.B.[156] have been reported to be of value. It should also be noted that an ineffective response to one steroid does not necessarily mean failure with other forms of steroid or ACTH therapy, or to a second course of the same steroid. The response to steroid therapy is variable and has been classified as follows: (a) complete remission with restoration to normal of all biochemical values (biochemical remission), (b) complete remission with residual proteinuria, (c) partial remission with loss of edema but with residual proteinuria, hypoalbuminemia, and hyperlipemia.

Soon after the introduction of steroid therapy, it was suggested that "maintenance" treatment be used because the relapse rate with this form of therapy was low and because antibody formation could be kept depressed. ACTH can be administered twice a week (1 mg. per pound of body weight) or given on 3 successive days of a week.[148] For maintenance therapy, steroids can be administered in doses of 300 mg. per square meter per day on 3 successive days of each week; a smaller maintenance dosage is sometimes effective. If a relapse does occur while the patient is on maintenance therapy, a full course of treatment equal to the initial therapy is again given, and possible reasons for the relapse are explored. Maintenance therapy has the advantage of causing less hyperadrenocorticism than that observed after prolonged continuous therapy. When the patients who receive initial short-term therapy only are compared with those receiving

the initial course followed by intermittent steroid therapy, the frequency of relapses is markedly diminished, resistance to steroids is less frequent, the survival rate is increased, and hospitalization is decreased in this latter group. Patients are followed closely for proteinuria and other evidences of relapse. Along with clinical evidences of improvement the inspection of the changes occurring in the kidney by serial biopsy study offer a guide to therapy and prognosis.[112]

There is no clear indication in patients who are in complete remission as to when therapy should be discontinued. Relapses have been reported 6 months to 2 years after cessation of steroid therapy.[157] It has been our policy to continue maintenance therapy for at least 6 to 12 months and then to reduce gradually the dose of steroid over a period of several months. One patient on 18 months of maintenance therapy relapsed. With reinstitution of continuous steroid therapy, this patient improved and the urine has remained protein free for the past 12 months. The reasons for these relapses are not clear; before the era of steroid therapy we had observed a relapse in one child 9 years after complete remission.

During maintenance therapy, it is agreed that antibiotic coverage is imperative in the presence of active infection. However, during the initial course of therapy, opinion is divided as to whether or not antibiotics should be given prophylactically. We have not used antibiotic therapy prophylactically because of the danger of development of antibiotic-resistant strains of bacteria in the patient and of development of drug sensitivity. With regard to the question of varicella, it is generally agreed that steroids should not be used during the invasive stage of the virus infection; following this invasive stage, however, there is disagreement as to whether or not steroids should be used. Gamma globulin has been suggested as a possible adjunct to therapy when a patient on steroid therapy is exposed to varicella. In preparation for operation, cortisone should not be discontinued.

To date, patients with infantile nephrosis have in general shown a poor response to steroid therapy.[158-162] The prognosis in this form of nephrosis is extremely grave and is far from approaching the estimated survival rate observed in childhood nephrosis, 70 to 80 per cent.[163]

In the treatment of the nephrotic syndrome the physician is much better fortified today than he was a decade ago because of the availability of corticosteroids and of therapy based on a more complete knowledge of the physiologic disorders of the disease. He is now justified in offering more hope for recovery.

We are extremely grateful to Dr. Ariadne Forbes and Dr. Manuel J. Esperanca for compiling the data for Figs. 2 and 1, respectively.

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The physician, the family, and the child with nephrosis

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NEW YORK, N. Y.

IN THE descriptive stage of medical science when knowledge concerning basic pathologic physiology was generally extremely limited and the etiology of most disease processes was unknown, a large portion of the effectiveness of the physician depended upon the doctor-patient relationship and symptomatic treatment.

As scientific knowledge concerning disease and its causation advanced, the other aspects of medical practice (art of medicine) received less attention, and because they have not yet been systematized into an organized body of scientific knowledge, they have even come into disrepute. Obviously, specific treatment of the underlying cause of a particular pathologic state takes precedence over the nonspecific, practical, and psychological measures that serve to comfort the patient and family. However, it should be almost equally obvious that it is not necessary to neglect the latter because the importance of the former has been recognized. In conditions, such as nephrosis, where direct prevention or elimination of the causal factor is not yet possible, the present-day physician is able to contribute to the welfare of his patients in only three ways: (1) He can utilize available knowledge to establish the diagnosis and to provide nonspecific therapy; (2) he can participate in relevant research activities; and finally (3) he can develop an awareness of the physical and emotional needs of his child patient as well as the patient's family, and attempt to be helpful and supportive to them. It is to these last-mentioned needs that the present section of this symposium is devoted.

Nephrosis and any other serious illness in a child act primarily as a nonspecific stress experience on the developing child and his family.[1, 2] Thus, the age and stage of development of the child, the latent sources of tension in the family, the personalities of the child patient and his parents will determine the psychological reactions to the child's illness to a larger measure than does the nature of the illness itself. In addition, there are certain specific features of any disease which have psychological implications for the developing child and his fam-

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ily. As the physician becomes aware of these factors, he can contribute more effectively toward fostering optimal development and adjustment in the child patient and his family and can help minimize disability and unnecessary anxiety and distress. These functions of the physician are particularly important in situations such as nephrosis where definitive treatment is not possible, and where prognosis for survival and future health is uncertain.

In the following discussion no attempt has been made to differentiate between the major problems presented by a child with nephrosis who is treated with adrenocortical steroids and those which existed before such treatment was widely used. In our opinion the problems which are most central to the psychological issues involved remain qualitatively the same. Two examples may serve to support this view. The first and most important is that, whether or not the proportion of children with nephrosis who ultimately survive is increased by the use of steroids, a significant number of treated children do not recover, and parents still have to face this ominous uncertainty. Second, there is no question that although treatment with steroids has diminished markedly, and in instances almost eliminated, the problems associated with massive edema, edema continues to be the principal clinical manifestation and when present it has profound physical and psychological implications. Finally, it is recognized that prolonged treatment with adrenocortical steroids presents additional problems, which will require consideration when sufficient experience with this treatment has been accumulated.

NONSPECIFIC EFFECTS OF NEPHROSIS ON CHILD AND FAMILY

With the impact of chronic illness, anxiety and guilt are the main emotional responses or feeling states that often are observed to be exaggerated both in the child himself and even more in his parents. Feelings of anxiety and a tendency to blame oneself for the occurrence of undesirable events are universal human traits. Appropriate and even less appropriate fears and feelings of anxiousness are easy to observe in all young children and even in most adults on slightly closer inspection. Children often reveal that they place the blame for various mishaps on their own misbehavior and the same tendency can be demonstrated in less naive and less outspoken fashion in many adults. These responses are brought to the surface in dramatic fashion even when minor illness occurs. Children will promise to be good in order to get well quickly or so that they may escape painful treatment procedures. Mothers when confronted with the superficially nonthreatening statement that their child has a diaper rash will leap to their own defense by reiterating how often they change their baby's diaper. If an illness is more severe and the parents are more disturbed, they tend to produce more far-fetched explanations for their child's illness, many of which implicate their own neglect or mismanagement. We have reported[1] group discussions with parents of children with nephrosis. In these discussions the parents showed a consistent tendency to assume the burden of blame for the child's illness. Many authors have cited instances of young children who attributed their own illnesses to earlier misbehavior, for instance, children with diabetes who felt they acquired the disease from eating too many sweets,[3] patients with rheumatic fever who thought they had brought on their condition by exercising too hard,[4] and even several children with nephritis who assumed that masturbation had precipitated hematuria.[4]

Besides adding to the distress of patient and family, the family's exaggerated feelings of anxiousness and self-blame in response to physical illness may lead to poor understanding of the disease, inappropriate behavior, and poor cooperation with medical personnel which may delay recovery. Not only can the physician who observes and understands these phenomena help the parents by relieving unnecessary anxiety and dispelling misconceptions, but he will profit himself because his contacts with the parents

will be less taxing and more rewarding. Also, he may avoid certain errors in his care of the patient such as veiled threats, "if you cannot do what I ask you, I can't be responsible for the outcome," punitive retroactive statements, "why didn't you call me when you first noticed the swelling?" and general authoritative instructions that do not take into account the parents' past practices, all of which may contribute to the already overwrought parents' distress and future anxiety.

There are many kinds of family problems that may be brought into focus and develop into major crises when a child develops nephrosis. The relationship between the father and mother as husband and wife may become strained or break down completely when tested by the many problems arising from the child's illness. We have observed a number of instances where even divorces appeared to be precipitated by this situation. Clearly, there were difficulties and dissatisfactions in these marriages prior to the child's illness, and the special demands placed on both marital partners proved more than the relationship could stand. The mother may not be able to satisfy her husband's emotional needs under the strain of special demands from the sick child, feelings of anxiety, pressures of practical and psychological nature relating to the child's illness and medical care. In other instances, a husband who has not been a source of great strength and support even under the usual conditions may prove himself completely inadequate to the mother in the more stressful situation. In the former situation, the appearance of the couple may suggest that the husband is jealous of the sick child. In the latter, one may get the impression that the mother is completely disgusted and discouraged with the husband as a responsible partner in the situation, "Oh, Sidney—it's no use talking to him. He won't be of any help."

It is worth mentioning another possible family reaction to severe physical illness in a child. Occasionally, it has been observed that the occurrence of nephrosis in a child acts as a constructive unifying force on family members. It appears as if a new solidarity results in response to the illness which is perceived as the common enemy to be fought by the family members together. In these instances, minor interpersonal problems and other preoccupations appear to be put aside and the energies of the family are concentrated on a common purpose.

Another source of family conflict has to do with the reactions of the siblings of the child with nephrosis. Exaggerated sibling jealousy develops easily when, for objective reasons, a large measure of the family attention goes to the sick child at the expense of the others. The well sibling may exhibit various kinds of disturbing behavior, and even somatic symptoms, in his quest to recoup some of the lost attention. At times, the family's preoccupation with disease, doctors, hospitals, and the general anxiousness may exaggerate latent fears concerning illness and death in the well child. The mysterious disappearances of the sick child to the hospital, aggravated by the fact that hospital regulations usually preclude any visiting by the siblings, may give the well child the uneasy sense that at any moment the sick child or anyone else including himself can be swallowed up by distant, invisible powers.

Latent problems in the parent-child relationship also may become manifest or more malignant in the illness situation. Most commonly, these are between the sick child and the mother. Even in the healthy young child during his preschool years mothers may find it difficult to allow increased independence to develop. Frequently, it is around the physical care of the child—feeding, cleanliness, toileting, etc.—that one sees evidences of mothers' inability to let children assume responsibilities for themselves at an age when developmentally they are ready to do so. The circumstances around illness lend themselves to an exaggeration of this tendency. The overprotective mother may seize upon the child's increased physical needs and passivity during illness and in the process of nursing may be able to keep the child closer to her and more dependent than would otherwise be possible. The more ambivalent her basic feelings toward the child are and the more

she blames herself for her own shortcomings as a mother, and perhaps for the illness itself, the greater will be her need to "make it up to the child" and to prove by martyr-like devotion that she really is a good mother. Particularly in a disease like nephrosis, in which complete recovery may occur after a long drawn-out illness, the effects on the child's subsequent personality development, involving especially his attitude toward his own body and about physical illness, may become a more severe handicap than are the effects of the illness as such.

The physician who knows or skillfully finds out about the family situation before the onset of the child's illness, who understands the personality of the parents, the emotional climate in the home, and the prevailing attitude toward illness and physical functions, will be alert to the emotional problems that may be encountered and can serve an important preventive, as well as supportive and therapeutic, function to the family.

SPECIFIC EFFECTS OF NEPHROSIS ON CHILD AND FAMILY

For the child who suffers from nephrosis, there are a number of specific psychological hazards in addition to the direct and reactive implications of any chronic illness.

Nephrosis is an illness in which, at times, the child suffers from severe malaise. Consequently, during these periods the child tends to be irritable, cranky, negativistic, and to behave less well than he is able to when he is physically healthy. This evokes adverse responses from the adults in the environment, be they parents and relatives in the home or medical personnel in the hospital. The child, who cannot differentiate between his own physical and psychological state, is unable to behave better, becomes more depressed as he is met with criticism and diminished warmth from the environment, and becomes less and less prepossessing to those who care for him. Thus, in addition to feeling miserable physically, he may feel that he is also a very "bad" child, and that he deserves to be punished. When he is confronted by painful procedures or unpleasant restrictions in activity, he feels that he is getting his just deserts for his bad behavior. All this fits in with the generally observed tendency of children, especially in the age group where nephrosis occurs most commonly, to carry a heavy burden of guilt feelings accompanied by fears of retribution. The usual age at onset of the nephrotic syndrome (between 1 and 3 years) is singularly unfortunate from a number of developmental viewpoints. This is the age period when normally muscular development and big muscle activity are in the foreground. Every pediatrician and all others who have had the opportunity to observe children between 1 and 3 years of age are familiar with their boundless activity and their irrepressible needs to run, climb, push, pull, kick, and throw. As the necessary physical strength becomes available, and as the growing child learns to control the parts of his own body, he clearly has a need to use them to the fullest to explore what he can do to practice his newly acquired skills. This serves a vital function in his development. The child with nephrosis often is physically limited in what he can do and, in addition, is curbed by the medical regimen as well as by his anxious parents. During periods of bed rest there is an extreme degree of limitation in the child's motility and muscle activity but even during periods of more or less complete remission he is unable to use his body the way he normally would and should. It is interesting that verbal expression may serve in some manner as compensation for this loss of physical activity.

Another developmental trend that is evident in growing children at the age when nephrosis commonly begins relates to the achievement of increased independence from the adults in the environment, especially the mother. It is during these years that the child first really becomes differentiated as a separate individual person responsible for his own physical care in control of his body and to a degree the master of his own fate. Feeding, dressing, toileting, and elementary hygiene become aspects of his daily routine that need no longer be done for him, but

that he can handle himself. The sick child often is deprived in some measure of this progression toward independence. Parents and medical personnel share in caring for the sick child's needs at home and in the hospital. Specific aspects of his care are overemphasized because of his medical needs and, therefore, assume undue importance. His privacy is intruded upon, specimens are collected, food is unpalatable, and forced upon him with little regard for his preferences or appetite. He is subjected to many painful, frightening procedures concerning which he has no choice and the reasons for which he cannot understand. In addition, a high premium often is placed by his environment on cooperation and compliance, and efforts at rebellion and self-rule are discouraged forcibly. Paradoxically, although submission is encouraged, discipline in the usual sense is often neglected. Since the adults in the environment feel guilty over the necessary restrictions, deprivations, and discomforts that the child must suffer, they cannot bring themselves to set limits for the child in areas where he may need them. Thus, the child is deprived of another growth experience and is "spoiled" rather than given the opportunity to learn some of the rules of conduct and principles that govern social living and which would be appropriately incorporated by him at his age and level of development.

Expansion of the child's social world from the immediate family group to include other families, relatives, friends, and finally perhaps a nursery school or kindergarten situation is another process which normally takes place in the age group under discussion. Because of the illness itself and because of fear of cross infection, anxious doctors and anxious parents tend to limit the nephrotic child's social life perhaps more than necessary. Here then is another deprivation, another learning opportunity that may be missed by the young child with nephrosis. His main opportunities to learn to play with his peers may be on the hospital wards. The only adults from whom he learns that there are other worlds besides his home and other ways of doing things than those prevalent in his family may be the medical personnel, and even with them his contact is limited. This makes for a constriction of his universe at a time when it should be expanding with the greatest rapidity.

Although his normal contacts outside of the home are limited, the nephrotic child may have to undergo a great many painful separation experiences in the course of his illness. Rather than venturing forth in the outside world with strong support from his parents and with the assurance of prompt return to their protective presence, he repeatedly may have the experience of being more or less precipitously abandoned in a hostile, strange environment (the hospital) where unfortunately, but frequently, his contacts with his parents are very sparse indeed. The adverse effects of this kind of separation have been discussed adequately elsewhere[5-10] but are mentioned here as an additional obstacle to normal personality development for the child with nephrosis.

As he learns to use his body in the course of development and as he becomes aware of the reactions of the environment to himself as a bodily presence and as a person, the young child forms the concept of his own physical self which has been referred to as the "body image."[11] This image is formed jointly from subjective perception of parts of the body and bodily functions and from the manner in which important figures in the environment respond to him and to his and their own physical functions. The nephrotic child is in danger of forming a greatly distorted concept of body image. There is the physical fact that with the waxing and waning of edema he gets fatter and thinner, tending to look slightly grotesque at the edematous times and thin and puny during the dry ones. There is the imposed limitation in the use of his body. There are all the vigorous procedures, catheterization, venipuncture, paracentesis, that he suffers at the hands of medical personnel. There is an excessive concern of parents and others in the environment over all of the child's bodily functions, such as the

volume of urine he produces. There is the absence or rarity of a sense of physical well-being, vigor, and fun in physical exhaustion. Thus, unless every opportunity and every possible normal outlet is seized upon to alter this trend, the child with nephrosis may come to feel that his body is a pretty miserable, distorted, uncomfortable, and unpredictable structure that requires weird machinations from the outside to keep it going and is capable of giving little pleasure to himself or anyone else.

In addition, it is generally recognized that children between the ages of 2 and 5 show a particular fear of bodily injury and of mutilation of any kind. Their overconcern with the slightest scratch, the excitement over a small quantity of blood seen to ooze from a minor cut, and the mad rush for the Band-Aid are familiar phenomena to all. Whether psychoanalytic explanations relating this overconcern to castration fear are accepted or not, it is universally agreed that the young toddler and preschool child in the course of normal development shows exaggerated fear of bodily injury and for practical purposes it suffices for the physician to be aware of this phenomenon so that he can understand some of the responses of the sick child to disease and to treatment procedures.

WHAT CAN THE PHYSICIAN DO?

Given this unfortunate combination of a sick child handicapped in some of the essential functions at a most vulnerable time in growth and development, an anxious family aware of the chronic course and doubtful prognosis of nephrosis, what can the physician do?

First of all the mature physician has usually developed a strong faith in the resources within human beings, in adults, and, especially, in children. In the practice of medicine one is constantly impressed with the way in which human organisms physically and psychologically are able to withstand even severe stress. A faith in this resiliency and resourcefulness can be transmitted in varied ways to patient and family and serves as a constructive force of great significance.

A sound knowledge of normal growth and development and of the emotional needs of children permits the physician to help the parents plan a program which will allow the sick child to compensate for many of the necessary deprivations resulting from the illness. Within safe limits the child should be allowed a maximum of motor activity. The parents can be encouraged to provide age-appropriate toys and other outlets for the child's need for mastery, activity, and growing independence. A conscious effort should be made to avoid doing things for the sick child which he is able to do for himself. Every possible occasion to give him a choice or to allow him to make a decision for himself should be capitalized upon. When decisions must be made for him, every effort should be made to help him understand why. When hospitalization becomes necessary, he should be given advance notice so that he will not feel he is the helpless victim of forces he can neither understand nor influence.

The physician should help parents recognize that body movement and activity are normal outlets for the child's aggressive feelings, and that these are not sufficiently available to the sick child. The parents may then understand better when the child uses other outlets excessively, such as verbal expressions of aggression. Such understanding may serve to minimize irritation on the part of the parents when they are confronted with evidence of aggressive feeling leading to expressions of exasperation, such as, "You really are impossible—no wonder you got sick," which will fit into the child's worst fears and fantasies. If the physician helps the parents to expect the child to get angry and difficult at times, almost in self-defense against all the nasty things that are done to him, they can extend instead a measure of empathy, "We know it's tough to be sick and not to be able to do what you want and to have all those needles and things. No wonder you feel angry, but everything that is being done will help you get better."

The most important thing for all adults who care for the sick child is to be aware of his possible reaction to the hardships he encounters. If the child's fears and disturbances are recognized, usually they can be alleviated, at least partially.

Procedures and painful treatments can be handled more tactfully if the physician utilizes advance warning when possible, simple explanations, and, when feasible, encouragement of the child's cooperation and participation. The child's independent powers of control can be utilized to help him master the situation and his fears. For example, he can be asked to hold still for a venipuncture or to cooperate in collecting a specimen. Even hospitalization can be an experience in increased independence if the child's cooperation is obtained and if his willingness to separate from the parents is appreciated as a sign of growth.

The physician also can help parents provide occasions for the child to extend his horizons beyond the hospital ward and his own room at home. Contacts with other children, learning experiences, preferably in a group, ventures beyond the confines of the home can usually be permitted in the course of prolonged illness, if, realizing their importance, the physician is able to relieve parental fears sufficiently and to educate the parents to the importance of the child's developmental needs.

The main obstacles to the creation of a wholesome milieu for the sick, growing child lie in the anxieties of the parents and of the physician especially, in the case of an illness as potentially dangerous as nephrosis. The physician, unfortunately, may be motivated not wholly by what is best for the patient and family but partly by fears that colleagues and the patient's family may blame him for having been negligent. It is true that it is very unlikely that a doctor will be accused of having been overcareful, and even more rarely will he be held responsible for emotional disturbances resulting from overprotection of a sick child. On the other hand, if he encourages nursery school attendance for a nephrotic child in remission, and such a child later develops an infection with an exacerbation of the underlying condition, the parents and others may ask themselves whether the child's course would have been more favorable had he been isolated at home. However, these decisions must be made by the physician on the basis of knowledge concerning both the natural history of the illness and the individual situation and the overall needs of the child. It is generally observed that the physician who knows the most about a particular disease such as nephrosis becomes more permissive in his handling of the patient, whereas the one whose experience is more limited tends to be more anxious himself and to transmit his anxiety to the family, and, therefore, indirectly to the child.

Besides possessing a knowledge of growth and development and of emotional needs of children and parents, the physician needs to have and to display an interest in the illness experience at it looks to the child, and especially to the parents. He may know that the urea clearance and urine specific gravity are more important indicators of the progress of the disease than are fluctuations in body weight and day-to-day variations in the child's food intake and activity level. But, the parents are concerned by what they can observe. In order to inspire trust and to be able to give effective reassurance to the parents, the physician must show that he knows what they are experiencing. If they do not sense that the doctor understands, they will not be able to accept his admonitions "not to worry."

When possible, it is also important for the physician to display a degree of empathy with the parents' less rational reactions to their child's illness. For example, there are moments when the care of a child with nephrosis becomes such a burden on the family financially, practically, and psychologically that they feel increasingly resentful. The resentment may be directed diffusely against the Fates, or more specifically at the medical authorities, or even at times against the child. These understandable negative and hostile feelings contribute to the parents'

sense of being inadequate, bad parents and lead to unreasonable "uncooperative" behavior. An occasional empathic statement from the doctor, indicating that any parent is entitled to and likely to experience this kind of resentment, may help relieve the tensions and restore the parents' faith in themselves and in their ability to handle the situation for the benefit of the child in cooperation with medical authority.

Even when the doctor is justifiably anxious, it is not always necessary or desirable for him to impose his anxieties on the parents. They are entitled to a fair presentation of the facts and to prognostic statements if they request them. However, since statistics mean little when applied to a single case, and since each patient constitutes an individual problem, the doctor is justified in selecting and timing the information that he transmits to the parents in light of the total situation and according to the parents' ability to understand and to cope with anxiety. Even the best informed and most sophisticated parents of a sick child need the support that comes from the physician's authority and his willingness to take responsibility and to make medical decisions for them.

On the other hand the parents need not be excluded from active participation in and planning for the child's care. It is common for parents to feel helpless when they first understand the full meaning that their child has nephrosis, and this feeling may be transmitted to the nephrotic child who especially needs to perceive the parents as strong supportive and protective figures to help him through his frightening experiences. To minimize this reaction the physician should encourage active participation by the parents where it is appropriate. He can guide and even challenge them to provide a therapeutic milieu, to plan appropriate activities, to work out a palatable yet adequate diet for the capricious appetite of the nephrotic child, and to make the hospitalization experiences less unsettling. He can recognize and acknowledge their competence and resourcefulness, support them in their own ideas, and show appreciation for the fact that they have a difficult task and are performing it well. This will help the parents alleviate their feelings of self-blame, and will offer a more constructive outlet for their need to be active. Otherwise, parents may be prompted to shop around from Dr. Peter to Dr. Paul, to try unsafe home remedies, or to engage in other destructive or senseless activities to the detriment of the child's care and to the annoyance of the medical personnel in charge.

SUMMARY

In summary, then, an awareness of the psychological reactions and needs of the parents of sick children and the developmental and emotional needs of the young child with nephrosis enables the physician to make a significant continuing contribution to the welfare and comfort of patient and family. The parents need to feel that they can trust the physician to make the best medical decisions for them and their child, and that his decisions are based on expert general medical knowledge tempered with specific, up-to-date information about child development and interest in their individual situation. They also need to feel that the physician trusts them as parents to help him to provide the best of care to their child. They need his guidance in providing optimal conditions for development and adjustment of their sick child in the hospital or convalescing child at home, from a medical, as well as psychological, point of view. They need the physician's support and reassurance in dealing with their own anxieties and mixed feelings, so that they can be as supportive and reassuring as possible in their relationship to the sick child whose attitudes about his illness, his treatment, and himself as a person will largely reflect the attitudes of his parents.

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Studies of the incidence of carriers of enteropathogenic Escherichia coli in a pediatric population

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CERTAIN serologic types of *Escherichia coli* have been implicated in the etiology of diarrhea in man, especially in young infants. Epidemiologic studies have also revealed these agents in the feces of asymptomatic adults and children. The purpose of this paper is to emphasize the prevalence of the asymptomatic carrier state, especially in infants under 2 years of age, and to question the validity of the diagnosis "enteropathogenic *E. coli* diarrhea" when the only available evidence is isolation of one of the specific strains of the organism from the stool.

MATERIALS AND METHODS

In the winter of 1960, cultures of the stools of several infants who developed diarrhea while in the Boston Floating Hospital revealed enteropathogenic *Escherichia coli* (hereafter designated E.E.C.). In an effort to ascertain the source of these organisms, rectal swabs were obtained from hospital personnel and asymptomatic infants. Because a surprisingly large number of these individuals revealed E.E.C. and many yielded multiple strains, it was decided to determine the carrier rate of these bacteria in children admitted to the hospital.

Rectal swabs or stool specimens were obtained from all patients during a 4-month period (Jan. 6 to May 3, 1960). This was carried out as part of the routine admission procedure. Specimens were placed directly into a bottle containing 50 per cent glycerin-saline preservative and were inoculated within 8 to 12 hours onto the surface of eosin–methylene blue agar plates, which were incubated at 37° C. for 24 hours.

After incubation, several typical *E. coli* colonies were suspended in saline and mixed on a slide with commercial *E. coli* typing serum. E.E.C. gave 4+ agglutination immediately in type-specific serum, but did not spontaneously agglutinate in saline. The following specific antisera were employed: 026:B6, 055:B5, 086:B7, 0111:B4, 0119:B14, 0124:B17, 0125:B15, 0126:B16, 0127:B8, 0128:B12. Because type-specific sera were not

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available toward the end of the study only polyvalent sera were used; this accounts for the large number of untyped organisms.

E. coli serotypes contain three antigens, O, K, and H. There are three types of K antigen, A, L, and B, all of which mask the reaction and must be destroyed by heat before agglutination in O antiserum takes place. All known enteropathogenic strains of *E. coli* possess the B type of K antigen. Unheated suspensions of this organism agglutinate only in OB* antiserum. The agglutination of *E. coli* by type specific (homologous) OB serum is considered a positive reaction. B agglutination is specific and there is no cross relationship with other OB types. The agglutination of enteropathogenic *E. coli* by homologous OB serum is specific.

RESULTS

Stool cultures were made in 1,078 consecutive cases. E.E.C. was isolated from the stools of 85 of these patients in the course of 88 admissions (3 children were readmitted to the hospital during this study). Fifty-five of the individuals yielded positive cultures when admitted and 8 revealed the enteropathogenic organisms within 24 hours. Cultures had not been carried out in the latter group when they first came to the hospital, but they were assumed to have harbored the organisms on admission. Twelve of these 63 patients had diarrhea either as or incidental to their chief complaint. Fifty-one children, 4.7 per cent of the total number studied, were asymptomatic carriers of E.E.C. when they entered the hospital.

Twenty-two patients with negative stool cultures at the time of admission began to harbor these organisms only after a varying period of hospital residence. Nine individuals in this group exhibited no diarrhea at any time during the period of observation. When these 9 patients are added to the other 51 free of intestinal symptoms, the incidence of asymptomatic carriage of E.E.C. in the 1,078 individuals studied was found to be 5.6 per cent. These data are summarized in Table I.

Cultures for E.E.C. in several asymptomatic patients became negative during the period of study despite the fact that antimicrobial therapy was not given. The fact that 59 of 85 children (69 per cent) with pathogenic *E. coli* in the intestinal tract were entirely free of diarrhea or other bowel abnormalities merits stressing.

Of the patients with positive stool cultures for E.E.C., 52 were male and 33 were female. The age distribution for this group, as well as for the 60 asymptomatic children, is presented in Table II. As can be seen, the ratio of asymptomatic carriers to the total in the group over 2 years of age (44/48 or 92 per cent) is much higher than that in the patients less than 2 years old (15/37 or 41 per cent).

The specific serologic type of E.E.C. was identified in 66 of the patients studied. In 31 (47 per cent), 2 or more strains were present simultaneously; 21 had no intestinal symptoms while the remaining 10 had diarrhea of varying severity (Table III). All of the 10 serotypes of pathogenic *E. coli* for which antisera were available were detected.

Table I. Isolation of enteropathogenic *E. coli*

	No. of patients	*% of total admissions*
Total admissions	1,078	100
Total positive cultures	85	7.9
Positive cultures on admission	55	5.1
Positive cultures within 24 hours of admission	8	0.7
Total with positive cultures on admission*	63*	5.8
Diarrhea in group with positive cultures on admission	12	1.1
Asymptomatic carriers on admission	51	4.7
Positive cultures developed during hospitalization, but patients remained asymptomatic	9	0.9
Total asymptomatic carriers	60	5.6

*Patients from whom no culture was taken on admission but whose stool cultures within 24 hours after admission showed E.E.C. were assumed to have harbored these organisms on admission.

*The OB antisera used in this study were obtained from the Baltimore Biological Laboratory, Baltimore, Md.

Data relative to the frequency of occurrence of each type, present alone or together with others, are presented in Table IV. It is noteworthy that the number of serotypes isolated from single fecal specimens in individual patients varied from 1 to 7. Stool cultures in several children showed variation in the number of specific strains of E.E.C. present at different times. For instance, one youngster who was harboring 7 types of the organism at one examination was found to have only 1 type in the feces 2 days later. This explains the fact that, while Table IV indicates that 36 specimens revealed a single strain, a statement above indicates that only 35 patients yielded a single serotype. The most frequently isolated strains of E.E.C. were 0127:B8 and 0111:B4. In 7 stools, 026:B6 and 0111:B4 were isolated simultaneously while 0111:B4 and 0126:B16 were present together on 5 occasions. No etiological significance could be attached to the presence of multiple serotypes nor could any correlation be established between diarrhea or the asymptomatic carrier state and a specific serotype, present alone or in company with other types.

No strains of Salmonella or Shigella were isolated from the feces containing E.E.C. Coagulase-positive *Staphylococcus pyogenes* (var. aureus) was recovered from 7 patients who were harboring pathogenic *E. coli* at the same time; 4 of these children had diarrhea while 3 were entirely free of symptoms.

Fourteen patients with diarrhea were treated with neomycin orally in a dose of 50 mg. per kilogram per day, with variable results. None of the asymptomatic carriers were given any antimicrobial agent.

The great majority of the patients from whom E.E.C. was isolated came from eastern Massachusetts, the area from which most cases were admitted to the Boston Floating Hospital. There was no predominance of children with positive stool cultures from specific localities within this larger area.

Table II. Age of patients with E.E.C. in the intestinal tract

Age	*All patients with positive cultures*	*Total asymptomatic carriers*
< 6 months	24	9
6 months to 1 year	5	3*
1 to 2 years	8	3
2 to 3	7	5
3 to 4	6	6
4 to 5	8	7
5 to 6	6	5
6 to 7	3	3
7 to 8	3	3
8 to 9	4	4
9 to 10	1	1
10 and over	10	10
Total	85	59*

*This includes one patient who became an asymptomatic carrier during the first admission and was later readmitted as an asymptomatic carrier.

Table III. Simultaneous occurrence of more than one serotoype of E.E.C. in stools

No. of strains	*Patients*		*Total*
	Symptomatic	*Not symptomatic*	
2	4	13	17
3	4	4	8
4	1	2	3
5	0	0	0
6	0	2	2
7	1	0	1
Total	10	21	31

Table IV. Frequency of occurrence of each serotype of E.E.C. alone and with other serotypes

Strain	*Alone*	*With other serotypes (No.)*						*Total*
		1	*2*	*3*	*4*	*5*	*6*	
026:B6	–	8*	–	2	–	–	–	10
055:B5	1	2	1	1	–	1	1	7
086:B7	1	–	4	3	–	1	1	10
0111:B4	9	12†	1	–	–	1	–	23
0119:B14	3	–	–	2	–	1	1	7
0124:B17	1	1	–	–	–	–	–	2
0125:B15	6	1	3	2	–	2	1	15
0126:B16	1	7‡	3	4	–	2	1	18
0127:B8	13	2	5	4	–	2	1	27
0128:B12	1	3	5	2	–	2	1	14

*7 with 0111:B4.

†5 with 0126:B16; 7 with 026:B6.

‡5 with 0111:B4.

DISCUSSION

Most studies dealing with the isolation of E.E.C. from feces have involved epidemics in nurseries or sporadic instances of diarrhea in children. In the course of these investiga-

tions, the presence of a widely variable incidence of asymptomatic and convalescent carriers has been noted.[1] Data obtained from careful examination of large numbers of stool specimens led Gamble and Rawson[2] to conclude that, "It seems probable that pathogenic strains of *E. coli* come and go as part of the normal adult intestinal flora, and that, at any one time, about 1 or 2 per cent of the population are excreting them." That the presence of these organisms in the stool need not be associated with clinical disease was stressed by Neter and his co-workers[3] who studied a 1-week-old infant who was asymptomatic in spite of the presence of large numbers of E.E.C., Type 0111:B4, in the nasopharynx, throat, and feces. Stulberg and associates[4] found that in most cases of diarrhea associated with the recovery of Type 0127:B8, the organism reappeared in large numbers in the stool without recurrence of symptoms. Two infants with considerable growth of Type 0111:B4 in the stools in the absence of symptoms have been described by Shanks and Studzinski.[5] One asymptomatic adult carrier was detected in a group of 82 people in contact with infants during a hospital outbreak of diarrhea by Cooper.[6]

Bernet and colleagues[7] studied 128 babies during an epidemic of diarrhea presumed to be due to *E. coli,* serotype 0126:B16, and found one healthy 3-month-old infant who was excreting the same organism. Two of 8 attendants included in this study were noted to be asymptomatic carriers. The incidence of serotype 0111:B4 in the stools of asymptomatic controls was found to be as high as that in infants with diarrhea by Cathie and MacFarlane.[8]

Only a few investigations involving a survey of a general or asymptomatic population for the presence of pathogenic strains of *E. coli* have been recorded. In a study of patients on an obstetric service, Cooper and coauthors[9] noted a 12.5 per cent incidence of asymptomatic carriers. Cultures of vaginal and rectal swabs obtained from 361 women hospitalized for delivery of a child disclosed E.E.C. in 46 cases; only one individual had diarrhea. Twenty-three of 355 infants born to these mothers were found to be harboring E.E.C. in their feces but only one had diarrhea: the asymptomatic carrier rate in this group was 6.2 per cent. In an effort to determine the comparative frequency of enteropathogenic *E. coli,* Salmonella and Shigella, Cooper and his co-workers[10] carried out cultures of rectal swabs in 2,865 hospitalized children. E.E.C. was detected in 188 instances: no symptoms were present in 8 per cent. The incidence of asymptomatic carriage of pathogenic *E. coli* in 1,000 cultures carried out in 172 healthy youngsters was found to be 1.3 per cent by Cooley and Schliessman.[11] Curtin and Clifford[1] collected stools from 121 infants (birth to 6 weeks of age) admitted to a nursery for newborn infants for reasons other than intestinal disease. Two babies were found to be carrying pathogenic *E. coli;* both were less than 1 week old and had no diarrhea.

Pathogenic *E. coli* was isolated at one time or another, from the stools of 20 of 111 normal babies (living at home and seen for routine checks at a clinic) by Thomson, Watkins, and Gray[12]; feces were cultured every 2 weeks but on only one occasion were 2 consecutive specimens positive. Only 8 of the 20 children had loose stools. Since E.E.C. was recovered 21 times in a total of 1,681 examinations, it appeared that approximately one per cent of infants less than 1 year of age were harboring this organism at any given time. *E. coli,* serotype D433 (also designated as 0111:B4) was isolated, on one or more occasions, from 17 of 60 babies living in a residential home by Payne and Cook.[13] Eleven of the patients had no symptoms, one had slight feeding difficulty, and 5 had diarrhea and vomiting. At one time, as many as 10 of the 15 infants in the home were excreting Type D433, but an outbreak of intestinal disease did not develop.

The detection of an over all asymptomatic carrier rate of 5.6 per cent in the pediatric population reported in the present paper is not surprising in the light of the results of studies by other investigators. The facts that 4.7 per cent of the children were harboring E.E.C., in the absence of symptoms, at the

time of admission to the hospital, and that 69 per cent of the total group and 41 per cent of those under 2 years of age yielded pathogenic *E. coli* on culture when they had no intestinal disturbances raise considerable question concerning the validity of establishing the causal relationship of this organism in any single instance of diarrhea. It is obvious that, in the absence of any other detectable enteric pathogen, the acute bowel disturbance might be due to a virus, for example, and this possibility might be overlooked if the child happened to be a carrier of E.E.C. In order to document the relationship of this type of *E. coli* to a sporadic case of diarrhea, it is necessary to establish the presence of a significant elevation of antibody to the specific serotype isolated from the feces.

A most striking finding in the present study was the large number of patients found to be carrying multiple strains of E.E.C. simultaneously, especially in the asymptomatic group (Table III). This phenomenon has been commented on only 3 times previously; in none, however, were as many strains isolated at one time as are reported in the present paper. Cooper and associates[9] detected 2 serotypes in 6 of 46 adults and in 4 of 23 infants harboring E.E.C. The simultaneous presence of 2 types of pathogenic *E. coli* was noted by Mackel and colleagues[14] in 2 cats and by Mian[15] in one animal. To our knowledge, the simultaneous isolation of from 3 to 7 serotypes of E.E.C. from a single fecal specimen has not been recorded previously. The association of multiple strains with an absence of any intestinal manifestations seems quite remarkable and suggests wide exposure to many serotypes with consequent immunization or great variability in the "pathogenicity" of different strains even of the same serotype.

A question may be raised regarding the possibility that, because rectal swab cultures may be inadequate for revealing fecal organisms in adequate numbers, especially in the absence of diarrhea, the observed incidence of the carrier state is low and might have been higher had feces been employed for bacteriologic study. This is probably so because Cooley and Schliessman[11] have questioned the adequacy of rectal swabs for the detection of E.E.C., and Thomson and coauthors[12] have pointed out that if pathogenic *E. coli* strains are outnumbered by other strains of this organism the presence may be overlooked and a stool culture considered "negative."

CONCLUSIONS

1. In a study of 1,078 pediatric patients, the carrier rate for enteropathogenic *E. coli* at the time of admission to the hospital in those with no signs of intestinal disease was found to be 4.7 per cent. Nine children, initially free of the organisms, acquired them during the period of hospitalization. The incidence of asymptomatic carriage of pathogenic *E. coli* was, therefore, 5.6 per cent.

2. Sixty-nine per cent of the whole group and 41 per cent of those less than 2 years of age from whom enteropathogenic *E. coli* were isolated had no evidence of intestinal disorder.

3. Forty-seven per cent of the patients from whom typable enteropathogenic *E. coli* were isolated harbored more than one serotype of the organism at the same time.

4. The frequency of the asymptomatic carrier state of pathogenic *E. coli* makes it impossible to attach causal significance to the isolation of strains of this organism in sporadic episodes of diarrhea.

We wish to express our appreciation to the Nursing Department of the Boston Floating Hospital and the staff of the Diagnostic Bacteriology Laboratory of the New England Center Hospital for their help and cooperation in this study.

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Toxic epidermal necrolysis

The scalded skin syndrome

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THIS condition was first described in 1956 in Britain by Lyell[1] and in South Africa by Lang and Walker[2]; Lyell suggested the name toxic epidermal necrolysis. Although the etiology is still uncertain, it warrants recognition as a distinct and striking clinical entity.

Death has occurred in about 30 per cent of the recorded cases, and, since administration of corticosteroids early in the course of the infection may help to prevent a fatal outcome, early diagnosis is of importance.

The damage, be it toxic or otherwise, is exerted almost exclusively on the epidermis, producing a variable degree of necrolysis of the epidermal cells with at the most minimal edema and periadnexal cellular infiltrate in the dermis.

The resultant skin changes closely resemble those seen after scalding and may affect up to 80 per cent of the body surface.

To date at least 9 cases of this condition in adults have been reported in the British and South African literature[1-4] and we should like to report therefore on the condition as it appears in children.

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CASE REPORTS*

Case 1. J. G. was a 3-year-old girl.

History. This little girl was admitted to the ward on April 8, 1959, having been perfectly fit except for an attack of measles 2 months previously. Two days prior to admission swelling and redness appeared in the skin around the eyes, lips, and ears, spreading the same day to involve the chest and back.

On the day of admission the skin of the trunk, face, and limbs was extensively involved in a red "scaldlike reaction" with loosening of the skin and the formation of flaccid bullae. By April 10, the loose skin had peeled off, exposing a raw, red surface which was extremely tender (Figs. 1, 2, and 3). She had an intermittent fever to a maximum of 104° F. from April 8 to 13. On April 13

*Cases 1 and 2 were shown at a joint meeting of the Sections of Dermatology and Paediatrics of the Royal Society of Medicine of London in May, 1959. Brief descriptions appeared in Proc. Roy. Soc. Med. 52: 1029, 1959.

there was a dramatic improvement, no fresh lesions having developed in the previous 24 hours. During the next few days a complete postscarlatina type of exfoliation of the skin took place with the formation of the occasional small blister. The skin has been normal and the child fit and well since April 24, 1959.

Laboratory investigations. On April 8, 1959, the blood count showed: hemoglobin 11.8 Gm. per 100 ml.; white blood cells 10,400 per cubic millimeter; neutrophils 78 per cent; lymphocytes 18 per cent; and monocytes 4 per cent. The erythrocyte sedimentation rate was 21 mm. in 1 hour.

A throat swab on April 8, 1959, disclosed "a few colonies of hemolytic streptococci and a moderate growth of *Streptococcus viridans* and coagulase positive staphylococci." Another on April 24 showed "heavy growth of *Streptococcus viridans* and coagulase positive staphylococci."

No abnormal cells were seen in the fluid from an intact blister. On culture, a few colonies of micrococci were grown. Virologic examination was negative.

Gram stain of a skin swab on April 8 showed occasional gram-positive cocci. On culture, a heavy growth of coagulase-negative staphylococci were seen.

Gram stain of the eye swab showed debris only. On culture, there was a heavy growth of *Str. pneumoniae*.

On April 16 a culture from the nose showed a heavy growth of coagulase-positive staphylococci.

The serum protein was 6.1 Gm. per 100 ml. Electrophoresis gave a normal pattern.

Electrolytes on April 12 were as follows: plasma bicarbonate, 53 vol. CO_2 (24 mEq. per liter); plasma chloride, 613 mg. per 100 ml. (105 mEq. per liter); plasma sodium, 308 mg. per 100 ml. (134 mEq. per liter); plasma potassium, 16 mg. per 100 ml. (4.1 mEq. per liter); blood urea, 26 mg. per 100 ml.; calcium, 10.1 mg. per 100 ml.

The antistreptolysin titer on April 10 was 500 units per milliliter; on April 31 it was 333 units per milliliter.

Treatment. Prednisolone, 40 mg. daily, was given from April 8 to April 14, inclusive, with a gradual reduction in the dose until it was stopped on April 20; 330 mg. was administered. Tetracycline in doses of 150 mg. at intervals of 6 hours was administered for the same period of time.

The dramatic cessation of necrolysis in this little girl on the fourth day may be part of the natural course of the disease or may have been directly related to the administration of corticosteroids with or without the wide-spectrum antibiotic. It is perhaps of interest that when the child was allowed out of bed a few days after the steroid therapy was stopped, she developed blisters on the soles of her feet, suggesting that a weakness in the epidermis was still present, which required the additional trauma of walking and running about the ward to produce separation of the dermal layers.

A biopsy was not carried out on account of the severity of the illness, and it was thought that the clinical picture was sufficiently classical not to require histologic confirmation.

Case 2. J. B. was a 2-year-old girl.

History. She was admitted to the ward on Dec. 23, 1958, two weeks after having an upper respiratory tract infection with conjunctivitis and a generalized macular rash. This was treated with a cough mixture and oral penicillin for 5 days; no medicine or drugs were administered after Dec. 14, 1958. On December 21 she developed redness of the skin of the neck and by December 23 the skin of the entire body was extensively involved with erythema, widespread loosening, and the formation of flaccid bullae, which progressed to the development of large raw, red areas, the skin changes being identical to the appearance seen as a result of "scalding." By the end of the first week she was very much improved although a few small bullae continued to appear during the next week. A terminal universal postscarlatinal type of exfoliation occurred.

Five days before admission she had been scratched on the right cheek by a cat, the lesion being treated with an ointment containing neomycin and bacitracin.

Since her discharge from the hospital on Jan. 21, 1959, the child has remained well, and the skin has appeared to be normal.

Laboratory investigations.

A skin swab on Dec. 23, 1958, gram stained, showed debris only. Culture resulted in no growth. Another on December 31, gram stained, showed debris only. On culture, a heavy growth of coagulase-positive staphylococci and diphtheroids appeared.

Examination of the urine on two occasions showed no abnormalities; cultures were sterile.

The blood count showed hemoglobin 11.4 Gm. per 100 ml. White blood cells 23,600 per cubic millimeter with neutrophils 62 per cent, eosinophils 1 per cent, lymphocytes 34 per cent, and monocytes 3 per cent.

Treatment. Prednisolone was administered from Dec. 25, 1958, until Jan. 1, 1959, with an initial dose of 20 mg. daily for 3 days, and a total dose of 100 mg. Triamcinolone, 4 mg. daily, was given until Jan. 5, 1959.

Fig. 1*A*. Appearance of patient J. G. on April 10, 1959, showing extensive loosening and shedding of the skin.

Also Achromycin, 75 mg. every 6 hours, was given from December 24 to December 31; sulfamethoxypyridazine, 0.25 Gm. daily, from Jan. 1 to Jan. 5, 1959; and Chloromycetin, 250 mg. every 6 hours, from Jan. 7 to Jan. 11, 1959.

In this case the corticosteroid therapy was not started until some 4 days after the start of the condition and the sudden improvement noted in Case 1 did not occur. A gradual improvement ensued although active loosening of the skin continued to a variable degree for about 2 weeks.

Case 3. F. R., a 3-year-old girl, was admitted to The Princess Alice Hospital, Eastbourne, under the care of Dr. R. V. Harris, on April 24, 1958, and we are indebted to him for the following description:

Two days before admission she developed "grazes" and "places like scalds" on the skin of the chest and ears. She then became ill and feverish, and on the same day the face and lips became swollen. Her family doctor diagnosed tonsillitis and penicillin was prescribed. On the day before admission, the child cried all day and resented being touched. Flat blisterlike lesions appeared on the other parts of the body and the child was considered to be very ill and was admitted to the hospital. She had had no previous illness other than "colds" and earache during the previous winter.

On admission the temperature was 101° F., the child lying constantly in one position and crying if touched or moved. The face was swollen, and in addition there were extensive areas of loosening of the skin involving approximately two thirds of the body surface. Where the skin had peeled, red, raw areas were exposed. Fluid collected beneath the skin on the chest and back, forming flaccid blisters. The skin in apparently normal areas could be loosened by pressure of a finger. The tonsils were enlarged and inflamed, with surface exudate. Throat swabs yielded on culture *Staphylococcus pyogenes,* sensitive to all antibiotics except penicillin and streptomycin. Culture from a bulla yielded only *Staphylococcus albus.*

Fig. 1*B*. Numerous bullae and some shedding of skin on face and forehead (April 10, 1959).

The child was treated by a combination of ACTH gel (20 units daily) and tetracycline orally. The temperature did not return to normal for 9 days, although no new lesions appeared after 48 hours. The skin lesions healed without scarring, and she was discharged 14 days after admission as cured.

A few months later she developed another attack of acute tonsillitis without any skin changes.

Case 4. N. L. was a 4½-year-old girl.

This child was admitted to Llandough Hospital, Cardiff, under the care of Dr. J. Jacobs, on March 12, 1955. We are indebted to him for the following description:

She had developed a sore throat 10 days previously, and 2 days prior to admission red raised "pimples" were noted on the skin of the chest and neck; these soon spread to involve the skin around the eyes and mouth and the following day the skin began to peel from the lower back and the child complained of tenderness of the whole skin surface; she complained also of headache; there had been no known contact with infectious disease.

On admission to the hospital a "bullous type" of eruption was noted, involving the skin of almost the entire body, except for the legs below the knees; it was most marked over the face, behind the ears, on the trunk, and in the axillae. Both lips were swollen and cracked, and the tongue was strawberry colored. The temperature was 101° F. and Nikolsky's sign was positive. The hemoglobin was 80 per cent, the antistreptolysin titer was 200 units; cultures from the eyes and throat were sterile.

She was given Aureomycin and an electrolyte solution by mouth and tulle gras was applied to the skin, which was then covered with gauze and bandaged.

The child remained alert mentally, and the

Fig. 1*C*. Large flaccid bulla on wrist. Smaller ones on palm and tip of ring finger (April 10, 1959).

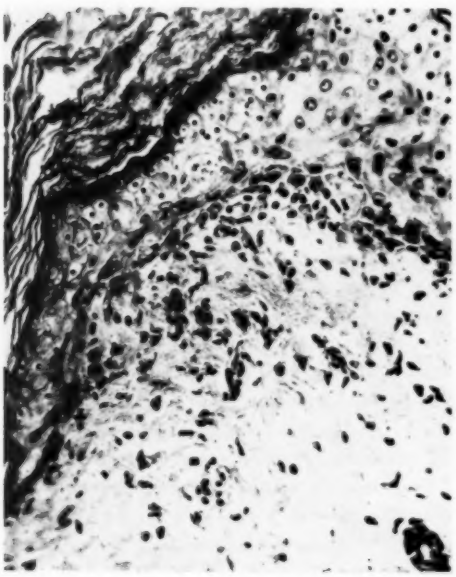

Fig. 2. Section of skin. Liquefactive degeneration in the stratum germinativum. (Hematoxylin and eosin. Original magnification ×225.) (From Lyell, A.: Brit. J. Dermat. 68: 355, 1956.)

Fig. 3. Section of skin with changes similar to those in Fig. 2, but also showing cleavage at the dermal-epidermal junction. (From Lyell, A.: Brit. J. Dermat. 68: 355, 1956.)

exfoliation increased. On the third day after admission she developed severe convulsions and died within 2 hours in status epilepticus, there being no clinical signs of dehydration. At autopsy no abnormalities were detected other than those of the skin.

Cases 5 and 6. Dr. Martin Beare has recently described two examples of this condition seen in his practice in Belfast. The first was that of an infant 2 weeks of age; the birth weight was 6 pounds and the delivery normal. At no time were any medicines or drugs administered. The infant was perfectly well during the first week of life and then suddenly developed a rash in both axillae which spread rapidly over the whole botly except the scalp, until he was denuded of epidermis, leaving a raw, red, weeping surface. The child died within 3 or 4 days of the onset of the eruption. Laboratory studies were noncontributory. The autopsy revealed no abnormalities other than partial pulmonary atelectasis and the extensive skin changes.

His second patient was a little girl aged 2 years, whose initial complaint was that of irritation of the skin, especially when put in a warm bath; one week later the skin of the axillae began to come away in sheets and within a few days the condition had become generalized. Her general condition remained good throughout the illness. The child recovered rapidly after the initial 3 to 4 days and within 10 days of admission was fit to return home. She has not had a recurrence. Again all investigations were negative except for a marked increase in alpha 2 globulin by electrophoresis.

Both these children were treated in much the same way with local and systemic antibiotics and in the first case a hydrocortisone lotion was also applied locally. Both were also given fairly large doses of corticosteroids by injection.

CLINICAL FEATURES

A variety of premonitory symptoms, such as vomiting, diarrhea, sore throat, may pre-

cede the development of the classical skin changes. In the case reported by Rook[4] recurrent attacks of the eruption followed on each occasion an attack of pain in the back. One of the children described here (Case 3) had had tonsillitis. Another (Case 2) had had a macular eruption associated with conjunctivitis and an upper respiratory tract infection and also had been scratched on the cheek by a cat. Case 1 had had an attack of measles 2 months previously.

Persistent high fever has been recorded, although this may be only of short duration. In the early stages fever may or may not be present. The temperature may return to normal within a day or two or persist for a week or more with varying degrees of severity.

The child may be extremely ill from the start or feel relatively comfortable with the first skin changes, and then suddenly progress to a highly toxic state which within a few days may end fatally.

The first skin change appears to be an erythema, perhaps with some degree of edema, resembling sunburn. This erythematous change may then rapidly spread over the surface of the body and merge with the next stage of loosening and separation of the skin. Fluid then collects under this loosened skin to form irregular flaccid bullae. Not uncommonly, extensive loosening of the skin appears without the formation of bullae. Within the next 24 hours the loosened skin will have started to separate and leave raw, red, denuded, oozing areas, sometimes involving practically the entire surface of the trunk and limbs. At all phases and even in the early stages, the child's skin appears to be extremely tender and he will resent any attempt at movement. This tenderness may even be present in the as yet apparently uninvolved portions of the skin. This combination of initial erythema and edema with irregular loosening of the skin, with or without flaccid bullae, followed by denudation leaving raw, red areas, produces an appearance, as Lyell[1] pointed out, identical with the effects of scalding of the skin.

The duration of the next stage is variable, and, if the patient survives, complete healing of the affected skin usually occurs within an average of 8 to 12 days. The cessation of necrolysis may be sudden about the third or fourth day as in Case 1, or it will gradually subside over a period of a week or 10 days as in Case 2. In both Cases 1 and 2, which we have personally observed, a universal postscarlatiniform type of exfoliation occurred soon after the healing of the previously denuded areas. As noted in Case 1 fresh blister formation may occur on sites of excessive friction such as the soles of the feet, if the patient is ambulant, and even after the condition has been quiescent for a few days. In both these cases, the hands and feet were the last areas to clear.

The severity of the affection is variable. In some instances it is of sudden onset with almost universal involvement of the skin, the patient is seriously ill, perhaps with a fatal outcome within the first 3 or 4 days. Mild attacks unassociated with any systemic upset have also been described, and therefore all gradations of severity between these two extremes may occur. It appears that the affection as it occurs in childhood is usually of the severe type.

Recurrent attacks have been reported in adults[1, 4] but as far as we know not in children.

There are usually no sequelae, the skin healing rapidly without scar formation. Lyell, however, reports on his fifth case,[5] "there is loss of elasticity over the buttocks; the nails have never grown again; the scalp hair took over one year to recover completely; the eyelashes are scanty; both eyes are affected by corneal scarring, the right being blind and the left giving appreciation of movement only; the buccal mucosa is thin and atrophic and there is some difficulty in swallowing."

Involvement of the mucous membranes has been reported, affecting the eyes, mouth, throat, and bronchial tree.[1-4]

The fluid from an intact blister in its early stages before secondary infection occurs is sterile, and tests for viruses in one case were negative.

HISTOLOGY

The damage is mainly confined to the epidermis and consists of a necrolysis of epidermal cells which in some areas may be complete, while in others it is patchy. The whole thickness of the epidermis may be affected or only an intraepidermal group of cells (Figs. 4 and 5). Acantholytic cells are absent. The dermis is usually relatively normal except for some vasodilation and edema. Lyell[1] was struck by the absence of changes in the sweat ducts in his cases, whereas there were signs of destruction in the sebaceous glands and their ducts; he wondered therefore whether the toxic substance might not be excreted in the sebum.

The occurrence of the histologic changes almost exclusively in the epidermis with minimal dermal changes differentiates the condition described here, as pointed out by Lyell, from the reactions of erythema multiforme, dermatitis herpetiformis, and bullous pemphigus and the nonspecific group of toxic erythemas. However, the possibility that toxic epidermal necrolysis occurring in the neonatal period and the so-called Ritter's disease are related or identical conditions is not clarified.

TREATMENT

Inasmuch as this condition of unknown etiology appears to have a high case-fatality rate, one is inclined to employ available drugs which may be beneficial in so-called toxic reactive processes, and especially if it seems possible that the condition has been initiated by an infection. Thus in some of these children corticosteroids and wide-spectrum antibiotics were given. In view of the rather protracted course in Case 2, large doses were given in the early stages to Case 1. The child's recovery was much more dramatic and sudden than in Case 2, although initially the child seemed more seriously ill and the skin was more extensively involved. It may well be, however, that these 2 cases represent natural variations of the disease.

SUMMARY

Six cases of toxic epidermal necrolysis occurring in small children are described. The condition, as it presents in children, is described and discussed.

We wish to thank Dr. R. T. Brain for Cases 1 and 2, which were admitted under his care at Great Ormond Street, and Drs. R. V. Harris, J. Jacobs, and Martin Beare for Cases 3, 4, 5, and 6, respectively; Dr. A. Lyell for the photomicrographs, Mr. Derek Martin for the clinical photographs, and the Board of Governors of the Hospital for Sick Children, Great Ormond Street, London, for permission to publish this paper.

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Chickenpox and leukemia

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SEVERAL authors[1-4] have noted the severe and often fatal nature of chickenpox occurring during the course of acute leukemia. However, no detailed description of this problem has appeared in the medical literature, nor have autopsy findings been reported.

In this publication, 4 cases of leukemia in which chickenpox developed are presented and discussed.

CASE REPORTS

Case 1. S. H., a $6\frac{7}{12}$-year-old girl, was first seen on Oct. 3, 1956, with a history of acute lymphatic leukemia diagnosed one month previously. She was treated with 6-mercaptopurine and Methotrexate with improvement, but because of toxicity 6-mercaptopurine was discontinued in November, 1956. She developed a partial hematologic remission in January, 1957, and continued taking Methotrexate, 2.5 mg. daily.

In March, 1957, she developed bronchitis and was treated with tetracycline. On April 5, 1957, she was exposed to chickenpox. On April 19 she developed fever, cough, emesis, and pallor. When seen on April 22, she had fever, three small vesicles on the skin, coarse râles in the chest, and hepatomegaly. During the next 3 days she developed increasing epigastric pain and emesis; more vesicles appeared on the skin and became hemorrhagic. Respirations became rapid and labored, the liver enlarged further, jaundice developed; the pulse became rapid and thready; body temperature became normal. She became restless and cyanotic despite oxygen, the blood pressure became imperceptible, cyanosis increased, and she died on April 25, 1957. She was alert, oriented, and talking until the instant of her death.

Blood count during her terminal hospitalization revealed a hemoglobin of 13.5 Gm. per cent and a white cell count of 4,100 with 48 per cent mature polymorphonuclear leukocytes and 52 per cent lymphocytes, some of which were immature. These findings fulfilled the peripheral blood criteria for partial remission. Chest x-ray showed numerous small nodular shadows in both lung fields.

Necropsy performed the day of death revealed generalized varicella and moderate leukemic infiltration of the bone marrow and kidneys. The skin had intraepithelial vesicles

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Supported in part by United States Public Health Service Grant CY-3900, and by funds contributed to Health Research, Inc., by the American Legion Auxiliary, Department of New York.

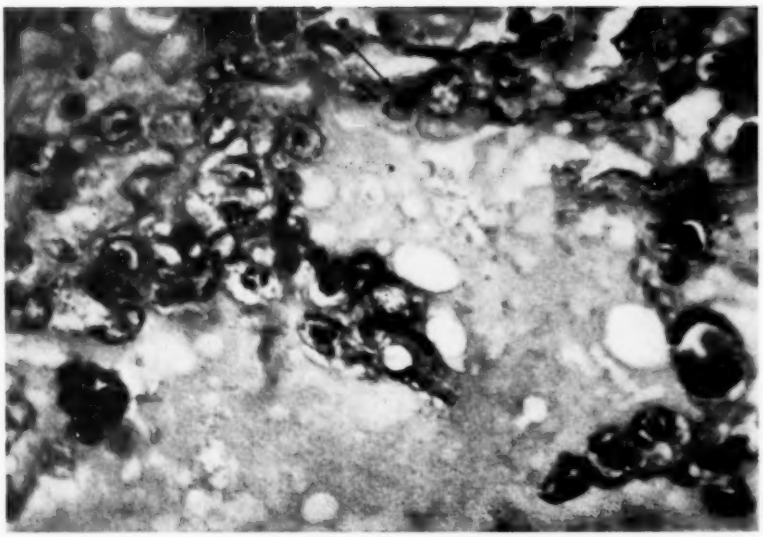

Fig. 1. Lung. Patient S. H. Note alveolar exudate almost devoid of inflammatory cells and intranuclear inclusion bodies in septal cells. (Original magnification ×900.)

with many of the epidermal cells containing intranuclear inclusion bodies. In the lungs (Fig. 1) there were pneumonic areas in which alveolar exudation and intranuclear inclusions of the septal and bronchial epithelial cells were evident. Focal areas of hemorrhage were also seen. The liver contained numerous areas of focal necrosis (Figs. 2 and 3 with hemorrhage but no inflammatory exudate. Many of the liver cells in these areas had intranuclear inclusion bodies.

Case 2. M. W., a 6-year-old boy, was first seen on July 10, 1958. He had developed enlargement of a cervical lymph node in June, 1958, and the diagnosis of lymphosarcoma was made by biopsy. Blood and marrow studies were normal, so x-radiation was given. After three treatments, however, he developed emesis and repeat blood count and marrow studies indicated that he had acute leukemia. Methotrexate therapy was initiated and he was referred to Roswell Park Memorial Institute. This antimetabolite was continued but treatment with prednisone was also started. In August he had a brief remission followed by relapse when prednisone was discontinued. Methotrexate was discontinued and in September 6-mercaptopurine was started. Complete hematologic remission ensued but in late October meningeal infiltration occurred as manifested by headache, emesis, papilledema, stiff neck and back, and spinal fluid pleocytosis. Intrathecal Methotrexate failed to produce relief and on Nov. 18, 1958, one x-ray treatment (75 r skin dose) was delivered to the area over the skull and spine. No improvement ensued, and when seen on November 23 he was in clinical and hematologic relapse with fever, fresh ecchymoses, papilledema, hepatomegaly, a white blood count of 68,400, predominantly lymphoblasts, and a platelet count of 30,000. Prednisone, 40 mg. daily, was restarted and 6-mercaptopurine, 50 mg. daily, continued. Subsequently his condition improved: he became asymptomatic, the white blood cell level fell to 13,500 per cubic millimeter, the platelets rose to 72,500 per cubic millimeter and the hemoglobin remained at 12 to 13 Gm. per cent.

However, on November 29 he developed abdominal pain and emesis and was hospitalized on November 30 for observation. On examination the temperature was 99.6° F. orally but the pulse was 140, and he seemed

lethargic and weak. The blood pressure was 140/70, there was bilateral papilledema, and the liver was palpable 5 cm. below the right costal margin. There was one small elevated red lesion in the scalp but the remainder of the skin was clear. Chlorpromazine, 7.5 mg., was given intramuscularly; prednisone, 7.5 mg. every 6 hours and 6-mercaptopurine, 25 mg. every 12 hours, were continued. That night he became increasingly drowsy and listless; he developed hematemesis and hematuria and more abdominal pain. The blood pressure rose to 150/110, and the liver became further enlarged to 7 cm. below the right costal margin. In the morning of December 1 lip ulcerations appeared and 6-mercaptopurine was discontinued, while ACTH, 20 units in 500 ml. of 5 per cent glucose in water, was started. Later in the morning small hemorrhagic vesicles appeared on the skin of the chest, abdomen, and leg, the temperature fell to 97.2° F. orally and the pulse became weak. At this point the mother discovered by telephone calls that a classmate who had brought him a gift from his school 14 days previously had come down with chickenpox the day after her visit. The patient became cyanotic, respirations became rapid, blood pressure imperceptible. A transfusion of fresh blood was started but at 2 P.M. he died. He was conscious, oriented, and talking until the moment of his death.

Laboratory studies on the morning of his death revealed proteinuria and hematuria, a blood urea nitrogen of 37 mg. per cent, and a uric acid level of 14.1 mg. per cent. Hemoglobin was 15.3 Gm. per cent, white blood count 20,400 per cubic millimeter with 35 per cent neutrophils, 2 per cent monocytes, 63 per cent lymphocytes and lymphoblasts, and 3 nucleated red blood cells per 100 nucleated cells.

Necropsy revealed intra-alveolar hemorrhage and edema in the lung with only a few leukocytes present. In the liver (Fig. 4) there were foci of hemorrhage and necrosis with many inclusion bodies, mostly intranuclear but also intracytoplasmic and even extracellular. The one epidermal vesicle studied contained giant cells. The marrow, lymph nodes, kidneys, testes, and meninges were infiltrated by leukemic tissue.

Case 3. R. D., a boy aged $4\frac{10}{12}$ years, was

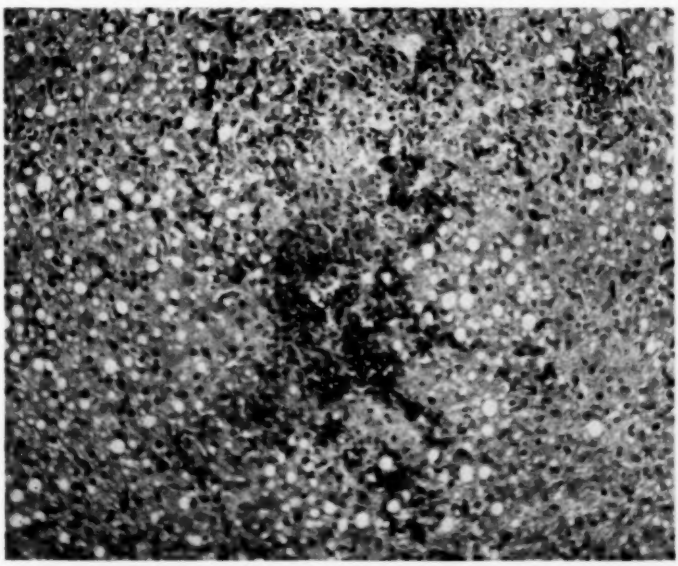

Fig. 2. Liver. Patient S. H. Note necrosis and hemorrhage. (Original magnification ×100.)

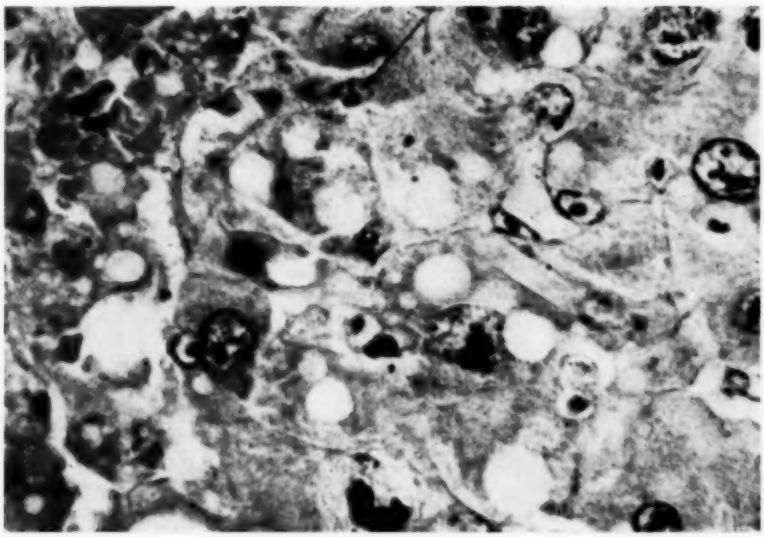

Fig. 3. Liver. Patient S. H. Note necrosis, lack of inflammatory cells, and intranuclear inclusion bodies. (Original magnification ×900.)

referred to Roswell Park Memorial Institute on Feb. 29, 1960, for treatment of acute leukemia. After an initial course of prednisone therapy, partial hematologic remission developed and 6-mercaptopurine administration was started while prednisone was discontinued. On May 2, 1960, hematologic and clinical relapse occurred with symptoms of bone pain and swelling of the hands and feet. On May 10 6-mercaptopurine was stopped and on May 11 treatment with dichloromethotrexate, 10 mg. daily intramuscularly, was initiated.

On May 12 fever up to 101° F. developed and on May 13 a vesicle on the occipital scalp was noted. On the following day more vesicles appeared on the scalp and neck and by May 17 there were similar erythematovesicular lesions in the mucous membrane of the mouth and over the skin of the entire body with the typical appearance of chickenpox. Dichloromethotrexate was discontinued.

Fever and eruption of fresh vesicles continued and hemorrhage appeared in some of the lesions. On May 20 the temperature rose to 103° F. and gamma globulin,* 40 ml. (6.6 Gm.), was given intramuscularly. On May 21 the temperature was 104° F. He was listless, complained of abdominal pain, and vomited. Another 40 ml. (6.6 Gm.) of gamma globulin was injected. By May 24 he was afebrile and the new lesions appearing were macular rather than vesicular.

On May 25 he developed fever again but no new skin lesions. Abdominal and extremity pain continued. Since no evidence was present of further activity of the chickenpox, it was thought that symptoms were due to leukemia in relapse and chemotherapy consisting of prednisone and dichloromethotrexate was started on May 30. He became afebrile the following day and gradually improved both clinically and hematologically. He was sent home on June 12 to continue taking prednisone and dichloromethotrexate as an outpatient.

During this hospitalization the hemoglobin

*Poliomyelitis immune globulin (human), Squibb, was supplied by Dr. Paul Yost, American National Red Cross, Washington, D. C.

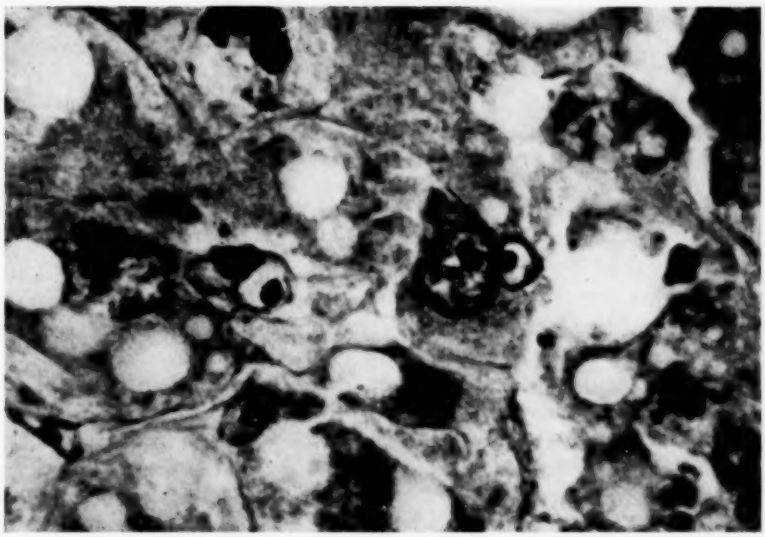

Fig. 4. Liver. Patient M. W. Note again the necrosis, intranuclear inclusion bodies, and lack of cellular response. (Original magnification ×1600.)

slowly fell from 11.6 Gm. per cent to 7 Gm. per cent on June 3; this was corrected by a transfusion of 500 ml. of whole blood. The white blood count rose from 3,600 per cubic millimeter at admission to 16,400 per cubic millimeter on May 30 and then fell to 2,200 after chemotherapy was restarted. Percentages of blast cells in the differential count paralleled the total white cell levels. The initial platelet level of 60,000 per cubic millimeter dropped to 12,500 on June 3 but reached 47,500 at the time of discharge. A bone marrow aspirate taken on May 11 revealed 84 per cent of the nucleated cells to be lymphoblasts.

Urinalysis showed moderate proteinuria on May 23 and 31. Blood, skin, and throat cultures on numerous occasions were negative for pathogens. Blood chemistry determinations including blood urea nitrogen, uric acid, bilirubin, alkaline phosphatase, and cephalin flocculation were normal. A lumbar puncture on May 31 revealed normal fluid and normal dynamics.

Antibiotics were administered frequently during hospitalization while the results of blood cultures were awaited. Penicillin, streptomycin, Chloromycetin, erythromycin, and Mycostatin were given at one time or another.

The patient died on Aug. 10, 1960, of leukemia in relapse and severe gastrointestinal hemorrhage.

Case 4. M. F., a white girl, was first seen on July 1, 1958, at the age of $3\frac{10}{12}$ years with a 4-month history of acute leukemia treated with 6-mercaptopurine and Methotrexate. Physical examination and blood and bone marrow studies indicated that she was in complete hematologic remission so the previous antimetabolite regimen of 6-mercaptopurine, 25 mg. daily, and Methotrexate, 1.25 mg. every other day, was continued.

On Oct. 30, 1958, she developed fever, cough, and pallor. Blood and bone marrow studies revealed relapse with predominance of lymphoblasts in marrow and blood smears. 6-Mercaptopurine was discontinued while the dose of Methotrexate was increased to 1.25 mg. daily. Clinical and hematologic improvement ensued but on Dec. 31, 1958, she

was brought in because a sibling came down with chickenpox on that day. Gamma globulin, 25 ml. (4.12 Gm.), was given intramuscularly divided among the gluteal and deltoid regions, and she was sent home to continue receiving Methotrexate. On Jan. 5, 1959, a lip ulceration appeared; Methotrexate was discontinued and another 10 ml. (1.65 Gm.) of gamma globulin was administered.

On January 12 Methotrexate was resumed in the same dose of 1.25 mg. daily. On January 19 she developed fever and evidence of pharyngitis. Blood count indicated complete hematologic relapse: the hemoglobin was 10 Gm. per cent, the white blood count 1,950 with 92 per cent lymphoblasts and lymphocytes, and the platelet count 10,000. Methotrexate was discontinued, another 10 ml. (1.65 Gm.) of gamma globulin was injected, and oral penicillin therapy was initiated. On the following day she was hospitalized for transfusion of 300 ml. of fresh blood. On January 21 she was discharged home in improved condition. Bone marrow examination on January 22 revealed almost complete replacement by lymphoblasts.

On January 24 a few erythematovesicular lesions developed on the face and scalp and on January 25 her temperature rose to 101.6° F. On January 26 she was afebrile and only a few healing skin lesions on the scalp, face, and upper trunk remained. No further evidence of chickenpox developed.

Her two siblings developed typical chickenpox on January 15; their lesions were more numerous and widespread but the courses of their disease were benign.

This girl subsequently received prednisone with some benefit and methylglyoxal-bis-guanylhydrazone, an experimental antitumor compound, without benefit. She died on June 7, 1959, with leukemia in relapse and generalized cryptococcosis.

DISCUSSION

The serious nature of chickenpox in children with acute leukemia is reflected by the courses of Patients 1, 2, and 3 of this series. In the first child progressive infection consisting of new skin lesions, pneumonia, and hepatitis continued for 7 days and terminated in circulatory collapse and death. The second child had a brief course: circulatory collapse and death occurred as skin lesions began to develop. In the third child, fever and new crops of vesicles continued for 12 days before evidence of active infection disappeared.

Necropsy on Patients 1 and 2 revealed focal hepatic necrosis and pulmonary edema and hemorrhage. Inclusion bodies were plentiful in both organs. Peculiar, though, was the lack of inflammatory cells in the visceral pox lesions which might indicate that these children suffered a deficiency in cellular response to infection. Silver and associates[5] have described defective leukocytic responses to bacterial infections in their patients with acute leukemia.

The reasons for the malignant course of chickenpox in patients with leukemia are not clear. Factual data concerning immune response to specific antigens in children with acute leukemia are sparse. Larson and Tomlinson[6] found that patients with acute leukemia had good antibody responses to pneumococcus capsular polysaccharides I and II. Other patients with this disease who were receiving cortisone had less response. Huang and co-workers[7] found that 10 of 18 children with leukemia receiving corticosteroids failed to form antibodies to Vi antigen. Of 12 children with other diseases receiving corticosteroid, 2 failed to respond. Skin hypersensitivity of the delayed tuberculin type and homograft response in leukemic children have not been studied. Their bleeding tendency, their infections and malnutrition, and their treatment with steroids, antimetabolites, and blood transfusions make controlled immunologic experiments in these children difficult to perform and interpret.

Adults with Hodgkin's disease are prone to develop serious and often fatal herpes zoster,[8] the clinical syndrome often considered a result of infection by chickenpox virus in the partially immune.[9] This may be related to their frequent deficiency in

"cellular" immunity, as manifested by delayed homograft rejection[10] and by loss of tuberculin sensitivity.[11]

Other than the leukemia itself, there is another possible reason for the severity of chickenpox in children with leukemia. In reviewing the literature of chickenpox, as of measles, one is impressed with the fact that no fatal cases of either exanthem in the course of leukemia were reported until recently. Bierman and associates[12] reported children with leukemia in relapse who developed chickenpox. Of the 3 for whom they supply detailed descriptions, none received chemotherapy during the incubation or febrile periods of their chickenpox and none became seriously ill with the exanthem. Two children had temporary hematologic remissions following chickenpox and in one of these the remission was prolonged by corticotrophin therapy, initiated after the patient became afebrile. The third child had a brief clinical remission. Wintrobe and his collaborators[13] described a child with acute leukemia in partial relapse who developed chickenpox while receiving small doses of Aminopterin. They did not report whether the chickenpox was mild or severe but mentioned that further remission of leukemia followed the infection.

These observations suggest that the agents used now to treat leukemia may have a causal role in the serious nature of complicating chickenpox. There is good evidence to support this concept. Cortisone and its analogues suppress host resistance to many infectious agents, including chickenpox virus.[4, 14] 6-Mercaptopurine inhibits antibody response to bovine albumin and prolongs skin homograft survival in rabbits.[15, 16] Methotrexate prevents mice from developing resistance to the virus of lymphocytic choriomeningitis.[17] All of the reported deaths from measles or giant cell pneumonia complicating leukemia were associated with treatment by one or more of these drugs.[4, 18, 19] Of the 2 fatal cases of chickenpox reported by Cheatham and associates,[20] one was that of a boy with generalized neuroblastoma who was receiving Methotrexate and the other of a girl with rheumatic fever receiving cortisone. Of the 2 children with fatal chickenpox complicating leukemia described by Nichols,[4] one was receiving prednisone and the other cortisone and Aminopterin. The case reported by Moulinier and co-workers[3] was being treated with cortisone. In the 3 serious cases reported here, one child was on Methotrexate therapy, the second was taking prednisone, and the third had recently completed a course of 6-mercaptopurine and was receiving dichloromethotrexate. Data concerning treatment of Toch's[1] and Gerard-Marchand's[2] patients are not available.

From available information, no relation can be drawn between severity of chickenpox and hematologic status in children with leukemia. The first patient of this series and the second of Nichols' pair apparently were in partial remission but both died of chickenpox. Patients 3 and 4 of this series were in complete relapse but survived. However, variations in type of leukemia and in therapy make comparisons impossible.

Weller[21] was unable to detect any neutralizing effect of convalescent serum in his early work with varicella virus in tissue culture. More recently he[22] reported partial neutralization of cytopathic effects in the presence of such serum in high concentration. Downie[23] confirmed this finding and pointed out that the neutralization test was more sensitive if fresh vesicle fluid were used as the source of virus. Since chickenpox is experienced by most people during childhood, one might expect that some neutralizing effect would be exerted by commercial pooled gamma globulin if used in sufficiently large doses.

Funkhouser[24] reported that human globulin protected children from chickenpox when administered in doses of 2 to 6 ml. within a day of exposure. Controlled studies by Schaeffer and Toomey,[25] utilizing doses of 5 to 10 ml., failed to confirm these findings. In Patient 3, 25 Gm. per square meter of body surface of commercial gamma globulin was administered during the course of the disease; defervescence and cessation of new vesicle formation ensued. In Patient

4, 8 Gm. per square meter of gamma globulin was administered on the day after exposure followed by 2.5 Gm. each week for 3 weeks. Despite the presence of leukemic relapse and Methotrexate toxicity, the incubation period of her infection was 25 days, only slight fever of one day's duration occurred, and about 10 to 12 vesicles developed in a 2-day period. This contrasted with the 15-day incubation period, longer duration of fever, and greater vesicle formation in her 2 normal, untreated siblings.

Although the significance of these observations is questionable at best, it would seem worth while to determine the possible usefulness of pooled gamma globulin in large doses for the modification of chickenpox. A study for this purpose in normal children is not justified because of the pain and the possible long-term dangers of parenteral administration of large amounts of homologous gamma globulin, and because of the benign course of chickenpox in normal children. But in patients with leukemia or generalized cancer who are receiving cortisone or its analogues, antimetabolites, alkylating agents, or large doses of ionizing radiation, who are prone to experience severe chickenpox and have a short life expectancy, such a study does seem justified.

The severe nature of chickenpox complicating leukemia demands that parents and physicians take special precautions against exposure of susceptible children with leukemia to varicella or herpes zoster. Since cortisone, certain antimetabolites, alkylating compounds, and radiation therapy are known to inhibit host resistance, physicians should consider the advisability of discontinuing or reducing the dosage of these agents when susceptible children with leukemia are exposed to chickenpox. The child in antimetabolite-induced remission usually can tolerate withdrawal of antimetabolite for 3 weeks or more without entering a relapse phase. On the other hand, it may be impossible to taper adrenal steroid therapy in the patient with relapse and bleeding tendency. These decisions must be made on an individual basis.

SUMMARY

This report concerns chickenpox occurring in the course of acute leukemia. Four children are described, including 2 who died with chickenpox and one who survived a prolonged course of the disease.

Histologic study in the fatal cases revealed focal hepatic necrosis and pulmonary hemorrhage and edema. There was a lack of inflammatory cells in the visceral pox lesions, suggesting a deficiency in cellular response to chickenpox infection.

The serious nature of chickenpox complicating acute leukemia may be due to the leukemia itself or to the drugs used to treat it. Adrenal steroids, 6-mercaptopurine, and Methotrexate all tend to inhibit immune response in certain situations.

Large doses of pooled gamma globulin administered to 2 children may have exerted some beneficial effect. Further trial of gamma globulin in large doses for modifying chickenpox in children with leukemia seems worth while.

Exposure of susceptible children with leukemia to chickenpox should be avoided. When exposure or illness does occur, the physician should try to discontinue or reduce antileukemia chemotherapy if it seems feasible.

Appreciation is expressed to Dr. John Pickren, Chief of Pathology, for reviewing histologic sections.

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TROPICAL PEDIATRICS

American trypanosomiasis

II. Current serologic studies in Chagas' disease

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IN 1909 Carlos Chagas described the first American case of trypanosomiasis in a human when he found, in the blood of a Brazilian child, the organism he called *Trypanosoma cruzi*. He had earlier discovered this flagellate in the intestine of a triatomiid bug. Tracing the life cycle of the trypanosome from animal reservoir hosts, via triatomiids, to man, he clarified the epidemiology of the disease. Subsequently, many investigators have added information on the distribution of *T. cruzi* in animals and insects in North and South America. Naturally infected triatomiids have been found in California, Arizona, New Mexico, and Texas.[1-9] In these same areas, wood rats, opossums, mice, and armadillos were also shown to be infected.[10-12]

Despite this association of mammal reservoirs and infected insect arthropod vectors, no instance of human infection was confirmed in the United States until 1955. In that year the first indigenous case was reported[13] and a second was certified to the public health authorities.[14] These cases showed that naturally occurring South Texas strains of *T. cruzi* were infective for man; they implied that unrecognized cases were probably present in the area; since both cases were in infants, they emphasized again that acute Chagas' disease is usually encountered in young children.

Earlier, it had been demonstrated that Texas insect strains of *T. cruzi* could be inoculated into primates[15] and into man[16] and Davis,[17] in 1945, sought evidence of unrecognized disease in humans by means of complement-fixation testing.[17] Using an

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This investigation was supported by P.H.S. Research Grant No. C-3338 (A) from the National Institutes of Health, United States Public Health Service.

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antigen prepared from cultures of a Panama strain, he tested 1909 serum samples and found one positive reaction. Significantly, the subject was an 8-year-old boy from a community near Corpus Christi, Texas, where the first documented case was later found. Although infected reduviid bugs were found in the child's house, the organism was not recovered by animal inoculation of the patient's blood.

Isolation of *T. cruzi* from the Corpus Christi patient led to a number of studies. The zoonosis of *T. cruzi* was reappraised and it was recognized that the habits of opossums and wood rats had changed in the last decade in such a way as to bring the animal-insect cycle closer to man. Clearing of land for farming and urban expansion has apparently forced these animals to move closer into man's settlements. They have become "suburbanites" along with the rest of us. Since the reduviids also share their new nesting places, the epidemiologic implications are apparent.[18]

Reports of animal studies indicate that the Corpus Christi human strain of *T. cruzi* is moderately virulent when compared to South American strains.[19, 20, 21] Other studies show host resistance to be lessened by such factors as malnutrition.[22] In South America a high reinfection rate is undoubtedly important in producing clinically recognizable disease; such reinfection is unlikely in the United States. These facts suggest that the disease pattern in the United States might be nondescript and not so readily recognized. Since it is notoriously difficult to recover the trypanosomes in chronic Chagas' disease, detection in this country must depend on something other than clinical recognition and recovery of organisms from acute cases.

Davis devised a stable and specific antigen from culture forms of *T. cruzi*[23] and de Freitas and Almeida[24-26] spent many years improving the antigen and establishing a precise and satisfactory complement-fixation test for Chagas' disease. Now that an antigen could be made from this (U. S.) human strain for use in this country, theoretical quibbles attendant on the use of a "foreign-strain" antigen could be avoided.

Widespread use of the complement-fixation test in South America has taught a number of lessons about Chagas' disease. It is still the only practical means for detecting chronic cases, for screening donors in blood banks, and for case-finding surveys.[27-35] Some results have been surprising. Kelser,[36] in Panama, showed an incidence of 3.7 per cent of unsuspected infection from a group of 1,626 persons by means of complement fixation. In El Salvador, a similar recent survey doubled the number of recognized cases.[37] Application of the test to prospective donors has also shown many unrecognized human infections; many reports indicate that the test reveals numbers of cases far in excess of those so far detectable by other methods.[27-35]

Experience in South America continues to reveal that, wherever the zoonosis exists, the incidence of human infection is higher than is generally realized and that acute infections produce rather nondescript clinical patterns which will not be recognized by clinicians who are not "trypanosomiasis conscious."[38, 39, 40] It was once believed that acute infection with *T. cruzi* was always followed by a chronic stage with severe visceral (especially cardiac) damage. Brumpt,[41] on the other hand, long ago maintained that chronic lesions were the result of reinfections leading to massive parasitization which overwhelmed the host's immune mechanisms. Case detection with complement fixation, together with studies of the parasite in animals, seems now to confirm Brumpt's stand, and to indicate that a moderate inoculum in a healthy child would produce a rather mild illness from which, unless reinfection occurred, recovery might well take place. This pattern should prevail in the endemic areas of the United States and could account for the past paucity of clinical descriptions.

The purpose of our present study is to evaluate the incidence of human trypanosomiasis in such an area by use of the complement-fixation test, which is the only practical means presently available.

PLAN OF THE STUDY

To accomplish this purpose, five criteria should be met:

1. That a proved and highly specific complement-fixation test be used.

2. That the antigen be prepared from a U. S. A. human strain of *T. cruzi*.

So far as human strains are concerned, it has not yet been conclusively shown that there is any marked antigenic strain specificity, at least in relation to complement fixation. Nevertheless, the use of an indigenous human strain in preparing antigen would seem desirable.

3. That children be tested. In South America the usual pattern is one of initial invasion in infancy with periodic reinfection throughout life, resulting in high titers of antibody over many years. In the United States, initial inoculation would also be more probable in the helpless infant but reinfection less likely. In this country antibody titers could fall with time and evidence of childhood infection be missed when only adults are tested.[42]

4. That sampling be done in an endemic area of high potentiality. The vicinity of Corpus Christi, Texas, would be a logical hunting ground. Animal and insect rates are high in the area[18] and the first native human case was found there.

5. That the children come from an economic group likely, because of their housing, to be in close contact with the animal-insect cycle.

METHODS

In order to meet these criteria, complement-fixation tests were performed on sera from 500 children coming to the clinics of the Driscoll Foundation Children's Hospital in Corpus Christi, Texas. This hospital draws its patients from the medically indigent within a radius of one hundred miles. In this region, four species of Triatoma have been found infected.[1, 5, 9] Opossums, chiefly in the towns and cities,[11, 43] and wood rats, chiefly on the farms and ranches,[11, 12] have been shown to be infected and to nest in and under dwellings.[18] The houses of these children, even in the towns, are usually of frame construction, supported off the ground on block foundations. This type of dwelling provides attractive nesting nooks for both opossums and wood rats.

The complement-fixation method employed was a modification of a precise procedure developed at the University of Sao Paulo, Brazil.[26] The antigen was prepared from the Corpus Christi human strain of *T. cruzi* according to the method of de Freitas.[26] It is stable for at least 6 months when stored at –5° C., and there were only slight insignificant variations of reactivity between different samples.*[25]

The hemolytic system consisted of a suspension of washed sheep erythrocytes colorimetrically adjusted to yield, when fully hemolyzed, an optical density between 0.52 and 0.60 at 530 mμ; these were sensitized with an optimal concentration of hemolysin. Guinea pig complement was titrated precisely, with the use of a plot of the complement volume and the per cent of hemolysis to determine the 50 per cent unit. Stock antigen was diluted 1:20 and titrated against 3 units and 6 units of complement, respectively. The primary reaction was carried out at 37° C. for 90 minutes; the hemolytic reaction at 37° C. for 15 minutes.

To save time, a qualitative test, using 3 units of complement and the appropriate dilution of antigen, was run on all sera. This detected the negative sera but did not differentiate anticomplementary sera nor those with low titers due to other causes. The reactive sera from the qualitative test were then subjected to a quantitative test. In this, various dilutions of serum were tested with 3 units and 6 units of complement in the presence of antigen, and undiluted serum was tested with 1 and 2 units of complement without antigen. Titers were calculated as a ratio of the amount of complement necessary for 50 per cent hemolysis when serum and antigen were present to the amount of

*Dr. R. G. Yaeger prepared the antigen used in these tests.

complement producing 50 per cent hemolysis with serum alone (Wadsworth's[44] formula:

$$T = \frac{D \times Ks,\ a}{Ks}).$$

Blood was drawn from unselected patients as they presented themselves for treatment at the Driscoll Foundation Hospital. After clotting, the serum was placed in sterile tubes with an identifying number and sent by air to New Orleans for testing. The sera were then refrigerated until the test could be performed. The 500 sera were identified only by the name, age, hospital number, and address of patients; no past or present medical data were available at the time of testing.

Accepting de Freitas' criteria, sera were considered positive if the titer was 1.9 or higher. He demonstrated that titers in the range 1.5 to 1.8 occurred predominantly in patients with Chagas' disease, but were occasionally seen in cases of leishmaniasis, leprosy, vaccinia, and syphilis. Eleven sera with titers in the 1.5 to 1.8 range were classed as doubtful and will not be considered further even though syphilis could be excluded and leishmaniasis or leprosy seemed unlikely.

The children with a titer higher than 1.8 were studied further. Exhaustive medical histories were taken and careful physical examinations were done. Blood counts, urinalyses, tuberculin tests, serologic tests for syphilis, protein electrophoreses, cardiac roentgenograms, and electrocardiograms were obtained for each one.

Isolation of trypanosomes was attempted. Blood cultures were planted on NNN medium. Direct or indirect xenodiagnosis* was performed, with 6 specimens of *Rhodnius prolixus*. The phytohemagglutination concentration test was carried out on each.[46]

Each home was visited in search of wood rat or opossum nests; triatomiids collected from these homes were examined for trypanosomes.

*A method of recovering *T. cruzi* from patients with the use of the insect vector as a culture medium. In the direct method, laboratory-reared triatomiids are fed on the suspect and the fecal droppings of the insect are examined for trypanosomes over the next 60 days. In the indirect technique[46] citrated blood is placed in a tube and a rat skin stretched over the mouth. The insects feed on the blood through the skin.

RESULTS

Blood samples were obtained from 500 individuals; all but 4 were children. There were 252 males and 248 females. The age distribution was: 0 to 5 years, 59; 6 to 10 years, 199; 11 to 18 years, 238; and over 18 years, 4. Four hundred and fifty-seven were of Latin-American lineage, 31 were Anglo-American, and 12 were Negro. One hundred and twenty-five lived on farms or ranches and 375 lived in towns or cities. Except for age distribution, these figures are representative of the usual proportions of these groups in the clinics of this hospital.

Seven sera from children were found to have titers of 1.9 or over. These served to indicate five family groups and blood was drawn from all members of these five families for examination. Two adults were found to have significant titers and are included. Thus, nine individuals out of 500 tested were found to have significant complement-fixing antibody for *T. cruzi*. Distribution as to age, sex, and lineage is given in Table I.

The contacts these individuals have had with the animal-insect zoonosis are summarized in Table II. All of them belonged to farm-worker families and had spent most of their lives on a farm or ranch. The houses in which they lived had been infested many times with *T. gerstaeckeri*, which is the predominant Triatoma in the area. All gave a

Table I

Case No.	*Age (years)*	*Sex*	*Lineage*
20	9	M.	Latin-American
44	4.5	M.	Latin-American
121	6	M.	Latin-American
228	14	M.	Latin-American
315	12	F.	Latin-American
390	15	M.	Latin-American
393	14	M.	Latin-American
399*	45	F.	Latin-American
411*	53	M.	Latin-American

*Secondary cases (see text).

Table II

Case No.	Residence	Insect infestation		History of bites	Animal nests		Infected insects
		Past	Present		Past	Present	
20	Rural	+	+	+	+	+	+
44	Rural	+	+	+	+	+	+
121	Rural	+	-	+	+	-	-
228	Rural	+	-	+	+	+	-
315	Rural	+	-	+	+	-	-
390	Rural	+	+	+	+	+	+
393	Rural	+	+	+	+	+	+
399	Rural	+	-	+	+	-	-
411	Rural	+	-	+	+	-	-

Table III

Case No.	Complement-fixation titer	Suggestive clinical data		NNN culture	Xeno-diagnosis	Phyto-hemagglu-tination	Serologic test for syphilis
		Past	Present				
20	2.1	Systemic illness pattern	0	0	0	0	0
44	2.0	Systemic illness pattern	Hepatomegaly, adenitis	0	0	0	0
121	2.0	0	0	0	0	0	0
228	2.0	+	Elevated serum gamma globulin	0	0	0	0
315	2.0	0	Elevated serum gamma globulin	0	0	0	0
390	2.3	0	0	0	0	0	0
393	3.4	Romana's sign	Elevated serum gamma globulin	0	0	0	0
399	2.5	Heart disease	0	0	0	0	0
411	2.2	Heart disease	Incomplete right bundle branch block	0	0	0	0

history of having been bitten on numerous occasions by such insects. All reported having had wood rats in or near their homes each year; opossums were known to have nested under two of the houses. Animal nests were found in or around the house in others. Infected insects were easily found at two of the five houses. At three homes insects were not present at the time of inspection (summer). This is probably because wood rats nest in the houses during the winter, bringing their insects with them; families reported that bites were more common during the winter months.

Clinical and laboratory data appear in Table III. Past and present medical findings are indicated. Six subjects had past illnesses which might, reasonably, have been acute trypanosomiasis. Five had findings at present, not otherwise explained, which might have resulted from such infection. From none of them were we able to isolate trypanosomes. All had negative tests for syphilis.

DISCUSSION

These results may be summarized: 7 individuals among 500 sampled from the vicinity of Corpus Christi, Texas, were found to have significantly elevated titers of antibody against *Trypanosoma cruzi,* 2 others were then identified in two of their households. From none of them could the organism be isolated. None showed any current physical or laboratory feature that could be rated as strong evidence of chronic Chagas' disease. Yet all lived in an area known to have a high rate of animal and insect infection with *T. cruzi.* All had been bitten by *T. gerstaeckeri,* which was recognized instantly by each mother and child as the locally

well-known *chince del monte* or "woods bedbug." Infected Triatomae were found on the premises of 4 of the patients. Wood rats were known to have nested in or about the houses of all. Opossum burrows were found on the premises of 2. Four of the 7 children had past histories of illnesses (documented by hospital records in 3 instances) which could reasonably have been the result of infection with *T. cruzi*. Six of the group have present clinical or laboratory findings which might be construed as resulting from chronic Chagas' disease.

Undoubtedly past and present clinical findings similar to those observed among these children would have been unearthed for any number of individuals whose complement-fixation tests were negative. Too much weight cannot be given to such recordings; neither can they be ignored. The difficulty of evaluating them stresses the protean nature of the signs and symptoms of Chagas' disease.

If one accepts the reliability of the complement-fixation test, the evidence seems adequate to warrant the conclusion that these 9 individuals have had past infection with *T. cruzi*. Certainly, no one would seriously question the clinical import of a quantitative Kahn titer of 1:320 in a 9-month-old infant, known to have been delivered of an untreated syphilitic mother and to have had a "runny nose" as a neonate. Few would withhold the diagnosis of syphilis, being willing to relent only if the *Spirocheta pallida* could be demonstrated. In so far as such problems of diagnosis are concerned, syphilis and Chagas' disease are comparable. In each, the causative organism is relatively easily demonstrated during the acute phases and becomes increasingly difficult to isolate as the disease becomes chronic. For each the diagnosis in the later stages is dependent upon a history of contact, a history of past compatible illness, suggestive physical findings, and a significantly elevated antibody titer demonstrated by complement fixation. Acceptance of a quantitative complement-fixation test in the diagnosis of syphilis is the result of confidence in the validity of the test, coupled with the difficulty of demonstrating spirochetes in the chronic phase.

Is there a similar difficulty in the isolation of trypanosomes from chronic cases of Chagas' disease? The answer is yes, especially in the United States, where reinfection is probably infrequent. Evidence comes from a comparison of epidemiologic factors here and in South America. In the latter area, triatomiid bugs infest the houses, are "domesticated," and feed primarily on humans. The dominant cycle is man-to-insect, and animals are rather incidental hosts. In the United States a sylvatic cycle takes place, with the parasite passing between wild animals and insects. Man becomes occasionally infected when the animals and their insects share his home. To illustrate the epidemiologic differences, use can be made of Dias'[49] report of finding over a 3 year period a total of 8,456 triatomiids in a single three-room rural house in Brazil. An average of 22 per cent of the insects examined (about one tenth of the total) were carriers of *T. cruzi*. With this number of insects, and year-around exposure to their bites, reinfection is certain to occur and positive xenodiagnoses on the 6 inhabitants is not surprising. In contrast, to find two dozen insects in a United States house is a bonanza and even this slight infestation would probably be seasonal.

Studies in South America with xenodiagnosis have shown a discouragingly low yield. In one instance, when 487 insects were allowed to feed on patients with known positive cases of Chagas' disease, only 62 of them became infected. When 20 insects per patient were fed on patients known to have parasitemia, *T. cruzi* was recovered from only one half the cases. When fewer insects were used in such tests (ten is about the highest practical number) yields became lower. These results were obtained in heavily endemic areas where reinfection was almost a certainty.[47, 48] Animal studies with the Corpus Christi strain of *T. cruzi* show that the flagellate disappears rapidly from the blood after massive inoculation. One must conclude that, in the absence of reinfection,

recovery of the blood form by any method is difficult in chronic cases.

Blood culture on NNN medium is less effective than xenodiagnosis; the phytohemagglutination concentration test promises to be somewhat more efficient. After reviewing the clinical and laboratory studies which bear on the question of parasite isolation from chronic cases, one tends to conclude that these methods are of uncertain value in proving primary infections after the acute stages are past. They are probably more valid for demonstrating reinfection parasitemias than they are as tools for detecting past infection.

How dependable is the complement-fixation test used in this study? When titers below 1.9 are excluded, experience demonstrates that false positives are extremely rare.[26, 27, 28, 31] If one takes the dimmest view of Davis' study and interprets the one positive result out of 1,909 tests as a false positive reaction, it is apparent that the test is not likely to lead to error in that direction. Various reports have indicated cross-reactions in cases of mucocutaneous leishmaniasis, but these have not occurred with titers over 1.8; no confusion has arisen with syphilis.

SUMMARY AND CONCLUSIONS

Nine out of 500 individuals tested in the vicinity of Corpus Christi, Texas, were found to have significant elevations of complement-fixing antibody to *T. cruzi*. Each was known to have been bitten by *T. gerstaeckeri;* the houses of 4 were found to be infested with insects carrying the trypanosome. In no instance could *T. cruzi* be recovered from the blood of these persons. The reasons for accepting this as valid evidence of past infection with *T. cruzi* are presented. These cases represent 1.8 per cent of the population sampled in this endemic area.

These findings indicate that infection of humans with *T. cruzi* is occurring in this area. They emphasize the need for physicians treating children in or coming from endemic areas to think of Chagas' disease in their differential diagnoses in order that acute cases of this illness may be recognized.

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COMMENTS ON CURRENT LITERATURE

Photic seizures—television-induced

MANY varieties of sensory, intellectual, and emotional stimuli seem to be capable of provoking seizures in specifically sensitive people.[1] The most commonly effective stimuli are visual, and of these the best known is flickering light. Evoked seizures were recognized by Gowers[2] in 1881 and fully described by him in 1901. Roman writings of the time of Galen describe this phenomenon, indicating that in sensitive persons a seizure might be provoked when a potter's wheel was rotated before the eyes. This means of distinguishing those potentially epileptic was used widely in Roman times as a slave-dealers' test and may well have been the earliest recorded association of convulsions with flickering light. Symonds,[1] who has made an extensive study of excitation and inhibition in epilepsy, stresses the fact that in some cases precipitation by a particular stimulus may be observed in only a small proportion of the seizures and may be omitted from the history so that the nature of the stimulus can escape notice easily. In his experience the element of flicker is not essential since abrupt transition from darkness to light, or the converse, may evoke a seizure in some instances. Two patients noted by Symonds[1] had difficulty on entering or leaving the cinema; another had trouble passing clothing over the head in dressing or undressing. According to Symonds, the "off," as well as the "on," signal from the retina may be effective. The same apparently holds true for auditory stimuli, as in the sudden transition from noise to unexpected silence.

Seizures resulting from flickering light have been recorded under widely varying circumstances such as blinking, waving the abducted fingers in front of the eyes while the subject is facing bright light or sunlight, driving down a tree-lined road against slanting rays, as of the setting sun, or walking near reflected fence railings. Modern developments have increased the forms of flicker to which the seizure-prone person is subjected: fluorescent lighting, neon signs, motion pictures, and television. Clinical observations of the effects of light are augmented by electroencephalographic investigations. Adrian and Matthews[3] were among the first to show that rhythmic changes in electrical potential could be recorded over the occiput when a subject's eyes were exposed to bright flickering light. Walter, Dovey, and Shipton[4] were able to evoke spike-and-wave discharges in an epileptic by photic stimulation, and many authors have confirmed that fits of all clinical types may be precipitated by photic stimulation. Some seizure-prone individuals have discovered how to employ light for fit induction in themselves. Such a case in a 7½-year-old girl was disclosed in 1960 by Whitty.[5] In this child the attacks were induced voluntarily,

and the compulsive nature of this behavior was quite apparent.

Considering the complexities of modern life and its attendant new forms of light reflection, the number of persons in whom seizures have been increased seems to be relatively small. However, in some instances television appears to be a hazard requiring careful consideration.[5] Five patients of this type who experienced television-induced seizures are reported in 2 articles which appear independently in a recent issue of *Lancet*. Two cases reported by Pallis and Louis[6] were in adults, one a woman and the other, a man. In neither family was there a definite history of epilepsy or other convulsive disorder. Neither patient had experienced head injury or encephalitis. The woman, a housewife 42 years of age, in good health at the time, had been under the surveillance of a physician because of a diagnosis of discoid lupus erythematosus made 9 years previously. In January, 1960, the family acquired a wide-screen television set which was difficult to adjust. This patient attempted to manipulate the controls while kneeling close to the screen. She remembers adjusting the controls and feeling intensely distressed; she lost consciousness abruptly, and her husband witnessed a major convulsive seizure. The patient was admitted to the hospital for observation. Both general and neurological examinations were entirely normal; the blood count, urinalysis, and the cerebrospinal findings were within normal limits. The skull radiographs were normal. The Wassermann reaction was negative.

Electroencephalographic studies were carried out the day following admission. Five baseline tracings were normal, "containing a regular symmetrical alpha rhythm at 8 cycles per second, which was readily blocked by opening the eyes or by any attention-provoking stimulus, including visual imagery. Overbreathing for 3 minutes repeatedly failed to alter the tracing."[6] Photic stimulation from a flickering source at frequencies of 18 to 30 flashes per second immediately produced fast regular symmetrical spikes followed by myoclonic jerking. This myoclonus proceeded to a major convulsion, if allowed to continue for more than a few seconds. Photic stimulation in the ranges, 3 to 18 frequencies per second and 30 to 40 frequencies per second, produced various combinations of generalized paroxysmal slow waves, spike-and-wave, and polyspike-and-wave complexes. At these flash frequencies rhythmic spike activity and clinical myoclonus were somewhat slower in developing than at the 18 to 30 frequencies-per-second level. No electrical or clinical disturbance could be evoked at 50 frequencies per second. At the optimum epileptogenic frequency, 20 frequencies per second, spikes and myoclonus could be evoked when the light source was held within 45 inches of the patient's eyes. At all distances between 3 and 40 frequencies per second, both spikes and myoclonus could be induced with greater ease and earlier when the eyes were closed than when they were open. At a distance of 40 inches and a flicker frequency of 18 flashes per second, the spikes and slow waves, produced when the eyes were closed, disappeared on opening the eyes and returned on closing them again. At all distances and frequencies the contrast between background illumination and flicker frequency was important. In a darkened room both clinical manifestations and electroencephalographic changes were evoked more readily. When this contrast was less, the stimulus could be tolerated for a longer period without untoward effect. "The susceptibility to all parameters of stimulation was extremely constant from day to day and at various times within a given day,"[6] emphasizing the specificity of the stimulus.

The second case reported by Pallis and Louis[6] is that of a man 27 years of age who was an interior decorator and a regular television viewer. The first episode in this case was not witnessed, but according to the patient himself, he must have been unconscious for a period of about 2 hours. The second episode occurred at home 4 months later. While adjusting the television set, he felt dizzy and suddenly lost consciousness. His wife witnessed a major convulsive seizure. The family history was noncontributory. On

admission to the hospital for study, the general and neurological examinations were entirely normal. The blood count, erythrocyte-sedimentation rate, cerebrospinal-fluid pressure and composition were within normal limits, and radiographs of the skull and chest were normal. The Wassermann reaction was negative. Electroencephalographic studies revealed abnormal resting records; the alpha rhythm was symmetrical, regular, and of moderate amplitude, with blocking on opening the eyes and during other attention-provoking stimuli. "It was interrupted by occasional paroxysmal bursts of symmetrical synchronous large-amplitude theta and delta waves, associated with irregular spikes and sharp waves."[6] Overbreathing resulted in an increase in the amount of paroxysmal activity. Photic stimulation in the frequency range, 9 to 30 frequencies per second, brought about paroxysmal abnormalities consistently. These disturbances varied from bursts of multiple symmetrical synchronous fast spikes at frequencies of 11 to 21 flashes per second to bursts of irregular spike-and-wave and polyspike-and-wave complexes at 22 to 30 frequencies per second. Generalized myoclonus began when spike activity continued for more than a few seconds. No abnormalities resulted at flash frequencies of 50 per second. Paroxysmal responses to flicker were evoked more readily when the eyes were closed than with the eyes open. While the range of effective frequency remained constant throughout the entire period of observation, in this patient, the distance and contrast aspects of effective stimulation varied from day to day.

Three patients with television-induced seizures are described by Mawdsley[7] in the same issue of *Lancet*. All 3 patients were female, one a woman of 34 years, and two girls 16 years of age. None of the three had a family history of convulsive disorders. The 34-year-old woman, however, had suffered for 5 years from attacks of migraine, sometimes precipitated by the glare of bright sunshine. In all three patients there was a striking association between their convulsions and watching television. One of the 16-year-old girls suffered 5 such attacks over a period of several months. All three patients showed marked electroencephalographic changes, and 2 experienced convulsions when stimulated with the stroboscope. In giving an account of their television experiences these patients described the flickering in similar terms, present on shifting of the picture or its disappearance from the screen. This light phenomenon has been explained on the basis of loss of synchronization between the signal and the time base, which involves a flicker frequency of about 25 per second. Because of the severe character of the convulsions, all three of the patients were placed on anticonvulsant drugs. Since the initiation of therapy, periods of 9, 18, and 14 months, respectively, none of the three patients has experienced a further attack.

Both these reports emphasize certain aspects of the problem of seizure excitation: that the incidence of clinically manifest epilepsy in the general population is high, and that latent susceptibility is more common than has been supposed.[8] Recognition of the part played by television was helpful in the management of these patients. All were advised to avoid adjustment of the set at close range, to have less light contrast between the room background and the television, to look away if shifting image or flicker is marked, and under no circumstances to remain facing the screen with closed eyes. Both studies stress the fact that television, especially when the set is out of adjustment, can provide a potent source of photic stimulation, and that, irrespective of family or individual history, persons who are extremely sensitive to light should avoid exposure to television flicker.

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Books

Projective Techniques With Children.
A. I. Rabin, and M. R. Haworth, editors, New York, 1960, Grune & Stratton, Inc.

This volume represents a description and an evaluation of the specialized application of projective psychological techniques with children. Projection in the sense developed by clinical psychologists refers to the presentation of more or less ambiguous stimuli to an individual to elicit his responses, perceptual and conceptual. The techniques thus allow a maximum of the idiosyncratic personality structure of the individual to appear for evaluation and tend to bypass, to an extent, the conscious defensive operations of the person. The techniques utilize a variety of stimuli: ink blots, pictures, toys, incomplete sentences, spontaneous drawings, free play, puppets, etc. They have been used primarily in psychological diagnosis of personality problems, though there is an increasing usage for research in personality theory, evaluation of the results of psychotherapy, and so on.

The book presupposes some acquaintanceship with the tests discussed and appears directed to advanced undergraduates and graduate students in clinical psychology. However, there is much of general interest to those concerned with personality theory. There is rich description of clinical experience, description of changes in personality structure with age, developmental norms in personality, and deviations from the norm. The interested individual who takes the trouble to acquaint himself with some of the special language of the tests (this would be primarily in reference to the Rorschach method) will find much of rewarding clinical value. In addition to description and evaluation of specific techniques, the volume also has chapters pertaining to the use of the techniques in research with children. Many of the chapters demonstrate considerable concern with concepts of reliability and validity and with developmental norms. They show an awareness of the methodologic problems presented by projective techniques and attempt to correlate research findings with clinical intuition.

The authors appear to have conceived their purposes in the various chapters in somewhat differing ways. Some emphasize the normative-developmental approach; others the reliability and validity of the specific instruments; a third group stresses the clinical utility, the meaningfulness of the techniques for understanding personality and its deviations. There is also a degree of variability in relation to the thoroughness with which a test or topic is discussed. This might be expected in a field where there has been so great a proliferation of techniques in a relatively short time. A few of the chapters are almost cursory, the majority, however, display clinical sophistication with considerable elaboration and thought given to the complexity of the subject. Over-all, the volume gives a clear picture of the present status of the techniques and their application to children. In addition, the bibliographies are quite extensive and facilitate further investigation of special interests.

WILLIAM J. MARK

Review of Medical Microbiology.
E. Jawetz, J. L. Melnick, and E. A. Adelberg, Los Altos, Calif., 1960, Lange Medical Publications, 376 pages. Price $5.00.

An adequate understanding of medical microbiology now implies knowledge in the areas of biochemistry, genetics, immunology, allergy, and metabolism as well as in the usual areas of bacteriology, virology, and mycology. While this paper-bound book does not pretend to answer all of the problems in this field it does provide a text which is concise, clearly written, and easily comprehended. Medical students, residents, and

"casual microbiologists" will find it a handy reference for the usual problems that occur in their contact with clinical infectious problems. In addition, more than 20 per cent of its pages are devoted to brief information in the fields of biochemical and genetic mechanisms applicable to microbiology. Adequate emphasis is given to the evergrowing volume of information in immunology and allergy. The bulk of the text is devoted to "classical microbiology," and here mention is made of some of the newly identified bacterial, fungal, and viral agents which produce clinical syndromes. The basic problems in virology are well discussed and provide capsule information which should stimulate investigation into the literature from which it was culled.

One of the disadvantages, acknowledged by the authors in the preface, is the lack of adequate bibliographic information.

As stated in the preface, the text is designed to provide "brief, accurate, up to date" medical microbiologic information. It makes no pretense at substituting for standard texts or at covering the vast microbiologic literature. It can, and does, provide a skeleton upon which the student of microbiology can affix much muscle.

V. A. FULGINITI, M.D.

Blood Diseases of Infancy and Childhood. Carl H. Smith, M.D., St. Louis, 1960, The C. V. Mosby Company, 572 pages. Price $17.00.

The field of hematology now includes morphologists, biochemists, geneticists, serologists, and followers of many other disciplines. The correlation of knowledge from these diverse fields and its application to pediatric hematologic problems has long indicated the need for a textbook on disorders of blood in pediatrics. The author has written a good, eminently practical text which should be of considerable value to all physicians who treat children. As is stated in the foreword, the book is written principally for students and practicing physicians. The hematologist will be disappointed if he seeks the intermediate metabolism of porphyrin ring synthesis, but the practitioner will find almost every pediatric hematologic entity well described, together with the usual methods of diagnosis and the preferred regimens of therapy. Controversial material is mentioned only briefly and the subject is presented in a most useful form. For those who may wish more information there is a quite comprehensive bibliography following each chapter.

It may be an indication of the direction in which hematology is heading that a text of hematology can be brought forth without the lovely, vivid, but seldom truly representative colored illustrations of cells which are usually included. Or it may be simply an indication of the rising cost of color reproduction and a laudable attempt to keep the price of the book reasonable. At any rate, there are no colored pictures in this book; in general, the black and white photomicrographs are poorly reproduced and add very little.

This excellent book would be much more useful for ready reference if the scattered tables and textual material regarding the normal values at various ages were gathered together in an appendix. It is hoped that this might even now be added in a second printing.

ARTHUR E. MC ELFRESH, M.D.

Fundamentals and Possibilities in Antituberculosis Vaccination. Richard Prigge and Gunther Heymann, Toronto, 1960, University of Toronto Press, 105 pages. Price $5.00.

This monograph by two German workers attempts to avoid the approach of most writers on artificially induced immunity to tuberculosis, by side-stepping the premise that the fundamental purpose is to test the efficacy of BCG immunization. Instead, the authors review much of the work on the immunology of tuberculosis and the immunochemistry of the tubercle bacillus. They discuss the three factors of resistance, immunity, and allergy in both experimental and spontaneous tuberculosis—and, as so many others have done who have explored these areas, they fail to arrive at any satisfactory conclusion as to the relative importance of these factors.

Essentially this book is a comprehensive review of available literature. As the authors point out, they draw no conclusion except that the information is still inadequate to evaluate the various methods of immunization which have been used in the past or are currently under investigation. In reference to BCG vaccination, they are inclined to believe that the present enthusiasm for this form of vaccination is unjustified, but believe that the critical clinical experiments are still to be performed.

WILLIAM J. MELLMAN, M.D.

Phosphorus Metabolism of the Brain.
P. J. Heald, New York, 1960, Pergamon Press, 195 pages. Price $6.50.

This is a monograph relating to metabolism of organic phosphate derivatives in the central nervous system. The author has divided the work into three parts.

In the first section in vivo metabolism is reviewed. The composition of the brain with respect to inorganic phosphate, adenosine triphosphate, phosphocreatin, and phospholipids is discussed. Quantities and metabolism of these components are affected by a variety of conditions. These conditions include convulsions, change in oxygen up-take, and normal development.

The second section is concerned with in vitro levels of phosphorus in tissue preparations of brain. Details of preparing brain for these studies are well described. Energy metabolism as indicated by changes in adenosine triphosphate and phosphocreatin are reviewed. Special emphasis is placed on the effects of intracellular and extracellular potassium and other ions, sedatives, electrical stimulation through special processes, acetylcholine, and some of the tranquilizing agents.

The third section is an appendix listing methods of analyses.

Though the book has a very narrow scope and though the author is frequently more concerned with quantities rather than physiologic effects, it is indeed an extensive review of much work relating to phosphorus and its components as related to the brain. The bibliography is extensive. This book will be of value to those interested in the energy relationships of the brain.

LEWIS A. BARNESS, M.D.

Correspondence

To the Editor:

Month after month, and year after year the vitamin advertisements in all the American pediatric journals reappear larger, more colorful, and more inviting—and yet over the past several years there has been an amazing paucity, if not virtual absence, of scientific articles in these same journals on this "crucial medicinal." In the year 1959, the vitamin sales in this country were in excess of $340,000,000, second only to the sale of antibiotics, and the trend over the past several years is steadily upward!

If one scans the advertisements one learns that, in addition to the conventional infant drops and pills, vitamins come also in the form of spheres (homogenized or otherwise) that can be chewed, or slowly dissolved in the mouth, or to be sprinkled on cereal, or in the form of chocolate nuggets, and can even be squirted in a "golden stream" from a pressurized can. One notes such phrases as "five delicious flavors," "delectable chewability," "fruit punch flavored," "melt in the mouth," etc., that make the reader feel he is perusing a confectionery trade magazine. Occasionally one sees a brief mention of assured or greater potency, but *nowhere* is there any mention as to *why* the vitamin supplement should be given.

This advertising is geared to the physician—but there are multiple sources of supply that range from the semiprofessional over-the-counter sale by the pharmacist to the mail order house. Most ludicrous of all is the actual retail "Vitamin Store" that does a thriving business in many parts of the country. Here a "pseudo doctor" will gladly diagnose your "deficiency" and "prescribe" a mixture to meet your "individual needs." The potential for hypervitaminosis syndromes in this situation is frighteningly real.

The implication is obvious. The pharmaceutical companies are not interested in the indicated use of vitamins in the treatment of avitaminosis or in legitimate supplementation in acute or chronic disease, malabsorption syndromes, etc., but are concerned with the daily "prophylactic" intake of vitamins by healthy children, eating for the most part well-balanced diets.

The major vitamins were discovered following observations of populations subsisting on grossly inadequate diets. It followed naturally, as their importance was established, that perhaps there existed subclinical vitamin deficiencies—and the daily vitamin supplement was born. It then followed that if a small pill was good, a larger one was better, and 2 or 3 large ones were even better; and thus from the infant drops to the adult pills to the geriatric high potency therapeutic capsule—Americans can boast the most expensive urine in the world.

Brief inspection of any nutritional text will quickly establish the fact that present-day recommended diets drawing from the 7 basic food groups (assuming commercial vitamin-D-fortified milk and bread) adequately supply all the basic vitamin requirements—and yet the vitamin binge continues to run rampant. The middle and upper class socioeconomic groups that buy vitamins for their children without a second thought are the very classes that provide ample nutrition for their families. The poorest classes from which the occasional case of avitaminosis arises can afford neither adequate food nor vitamins. In between one sees the not-infrequent poorer family spending a large part of their meager earnings on an expensive bottle of vitamins when the money might better have been spent on food that would have provided protein, calories (and vitamins).

The physician is to a great extent responsible for this situation. He uses vitamins as the universal panacea and placebo. Any patient, no matter what the complaint, can always be sent away safely with a bottle of vitamins. The mother who complains that her child is short and thin gets a vitamin tonic—a brief glance at

the mother may suffice to explain that the child's stature is inherited from the short, thin parent; both child and parent still nonetheless normal and healthy. The poor eater is given a vitamin supplement to increase his appetite, when a brief investment of time and discussion of the dynamics of his feeding problem might lead to a more basic solution. How often one hears the lament of the mother of a child with frequent upper respiratory infections who says, "I can't understand it doctor, I give him vitamins every day. . . ." I would venture that a physician has a better chance nowadays of seeing a case of vitamin toxicity than of seeing a case of avitaminosis.

The parents of the relatively disease-free, well-developed child on a daily vitamin supplement are frequently firmly convinced that the vitamins alone are responsible. The parents of the constitutionally thin, infection-prone child, and the one with a feeding problem continue nonetheless to give vitamins with blind faith lest they be criticized by some relative or neighbor for negligence.

Now that solid foods are being fed to infants at 2 to 4 months of age, and in view of the fact that all commercial milks are fortified with vitamin D, and hypoallergenic juices fortified with vitamin C are available, even the need for vitamins in early infancy can be questioned. But let the young doctor beware who suggests to a mother that her healthy well-fed child no more needs a daily vitamin supplement than he does a quart of milk a day. His competence may be questioned and the patient may seek a "wiser" physician.

One cannot help thinking that if the annual vitamin expenditure of $340,000,000 were spent to buy food for undernourished children and perhaps partly for nutritional education, a great humanitarian good would result.

At the present time, the widespread use of daily prophylactic vitamin supplements for healthy, well-fed children seems reprehensible on scientific, ethical, and economic grounds. The few editorial efforts along these lines[1, 2, 3] over the past few years seem to have been feebly lost in the sea of colorful pharmaceutical advertising. Inasmuch as it is the pediatrician for the most part that initiates the vitamin habit, it would seem appropriate for him to initiate some constructive reflection and action in this area.

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REFERENCES

1. Council on Foods and Nutrition: Vitamin Preparations as Dietary Supplements and as Therapeutic Agents, J. A. M. A. **169:** 110, 1959.
2. May, C. D.: Editorial, Pediatrics **23:** 833, 1959.
3. Sebrell, W. H., Jr.: Vitamins in Medical Practice, Seminar Report **3:** 2, Fall, 1958.

News and notes*

During 1960, the **American Board of Psychiatry and Neurology** certified the following persons in the subspecialty of **Child Psychiatry:**

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A **Postgraduate Seminar** in pediatrics will be presented by the Children's Hospital of the District of Columbia with the combined faculties of the George Washington and Georgetown University Departments of Pediatrics, and with a guest faculty also, from May 24 to 26, 1961. The general emphasis will be on information of value to the practicing pediatrician or general practitioner.

A one-year residency in **pediatric hematology,** beginning July 1, 1961, is announced at the Children's Hospital of the District of Columbia. The position, which is recognized by the American Board of Pediatrics, carries a stipend of $3,200 annually. Address inquiries to: Sanford Leikin, M.D., Hematologist, Children's Hospital, 2125 13th Street, N.W., Washington 9, D. C.

The Merrill-Palmer Institute of Detroit offers a workshop, **The Role of the Professional Person in the Racially Changing Neighborhood,** July 10 to 21, 1961. Tuition is $45.00; room and board can be obtained at a cost of $60.00; scholarships are available. Address Dr. Richard K. Kerckhoff, Workshop Leader, The Merrill-Palmer Institute, 71 East Ferry Avenue, Detroit 2, Mich.

The annual **Brenneman Lecture Series of the Los Angeles Pediatric Society** will be held at the Ambassador Hotel, Los Angeles, Nov. 8 and 9, 1961. Speakers will be Dr. Charles A. Janeway of Harvard and Dr. Robert Blizzard of Johns Hopkins. Address inquiries to Neil N. Litman, M.D., 9209 6th Avenue, Inglewood 4, Calif.

The American Board of Pediatrics, Inc. certified the following diplomates at Washington, D. C., Dec. 1 to 4, 1960:

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